ماجستير تناسليه (1)

Semen Analysis

د/هانى ابوالوفا

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just print

01025329200- 0502200362

Advanced Semen analysis

Optimal Tests

- CASA.
- Culture studies.
- Chemical "
- Chromatin "
- SCSA
- Sperm chromatin structure assay
- Sperm Chromatin
- Sperm

Sperm function Tests

1. Sperm mucous interaction
2. " - Capicitation.
3. Zona Binding & Acrosome reaction
4. Sperm ovum interaction. (SPA)

1. CASA

(Computerized Assesed Semen Analysis)
(Computer Aided " ")

def. Semiautomated Technique composed of Video Camera & Computer to visualize & Analyze Sperm Concentration & motility (Velocity & Kinematics).

Adv. ① Percision is high.
② Provision of Quantitative data on sperm Kinematics.
③ useful to detect effects of toxicants on sperm Kinematics & Morphometry (in Toxicology)

disadv. ① Expensive
② not accurate when Sperm Concentration is very Low or very high or when there are cellular debris.
③ hasn't shown to improve patient outcomes but rather, is helpful for research Purposes.

Parameters or data delivered by CASA are:

① Curvilinear Velocity (VCL): Velocity along it bet. 2 success.

② Straight line velocity (VSL): Velocity along its straight path bet. its 1st & Last positions (Measure For Forward progression)

③ Linearity: $\frac{VSL}{VCL}$

④ amplitude of Lat. Head displacement (LHD):

⑤ Average Path Velocity (.....)

⑥ Flagellar beat frequency

⑦ Evidence of Hyperactivation Motility ∠ LHD / VSL

large amplitude of Head & tail + ↓ progressive motility → Measurement of Hyperactivation

NB on LHD:

- dy: width of the path taken by head during movement.
- Significance: Critical width of path taken by sperm head needed For penetration of cervical mucous & oocyte envelope.
- The progressive motility is divided Acc. to the degree of LHD into 4 types:

- progressive motility with
  - minimal LHD
  - some LHD
  - Marked LHD
  - darting or whiplash LHD

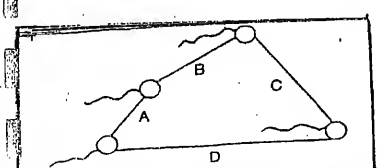

Figure 3-3: Calculating the curvilinear velocity.

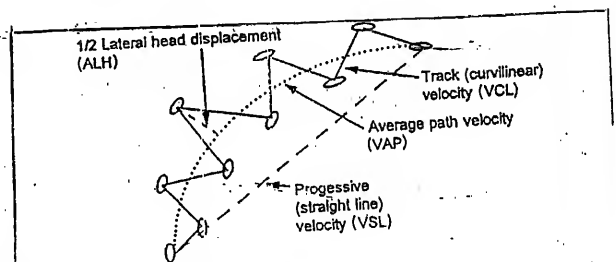

Figure 3-2: Principal paremeters of sperm motion meas'

So, CASA is valuable research tool than useful in clinical work!

2. Culture studies

may be needed for:

- **Semen**
  - if there is evidence of infection or inflammation
  - e.g Round cells (WBCs) > 1 million/ml or > 10/HPF
- **Urine**
  - if there is evidence of urethritis or cystitis, prostatitis.

3. Chemical studies

Seminal tests to study

- **Chemical markers:**
  - Epididymal markers.. (3) ?? mpi → α glucosidase
  - SV markers: (2) mpi → Fructose
  - prostate Markers: (4) mpi → Citric acid.
- **Sperm functions**
  - By estimation of "ROS" level
    - Origin:
    - Level:
    - Examples: NO, H_2O_2, Hydroxyl, Hydrolase

ROS (reactive oxygen species) & Infertility

- Def. of ROS: highly reactive oxidizing agents belonging to class of free radicals. a free radical is "any atom or Molecule that Possis one or More up paired electron"
- Types of free radicals:
  - H_2O_2 : Hydrogen Peroxide
  - .OH : Hydroxyl Radical
  - $.O_2^-$: Super-oxide anion

  ROS ← Reactive Nitrogen Species (RNS)
- Sources of ROS
  - WBCs (main source)
  - Sperms (NL & abNL)

Effects (Functions) of ROS

Beneficial Effects:

Small amount of ROS can regulate

Harmful Effects

High levels of ROS → Oxidative Stress & may → Sper

Natural Defence Mechanism against ROS
(ROS Scavengers or antioxidants)

- **Enzymatic antioxidants:** (مضادات الأكسدة الإنزيمية)
  - Catalase
  - Superoxide dismutase (SOD)
  - Glutathion peroxidase. (GP)
- **Non Enzymatic antioxidants**
  - Vit E
  - Vit C
  - Uric acid.

the Scavengers are present in Semen in specific level. if the balance bet oxidant (ROS) & anti oxidant (Scavengers) disturbed ⟶ oxidative stress.

- **ROS & infertility:**
  - in fertile Men ⟶ ver low amount of ROS
  - in infertile Men (25%): ⟶ High level of ROS ⟵ usually caused by leukocytospermia.

- **Lab assessment of ROS:**

4. Chromatin studies

(NL sp) (Sperm DNA Damage)

NL DNA of Sperm Means:

- No DNA fragmentation, oxidation (Nor) denaturation.
- Chromatin is: Stable or Condensed (NL Packaging)
- Histone replaced by Protamine (NB < NL Cells: Histones / Sperm: Protamine)

So Sperm DNA damage Means: DNA Fragmentation / Abnl Chromatin Packaging &/or Protamine deficiency.

→ Failed union bet ♂ & ♀ Gametes → No fertilization

[Sperms of infertile man have DNA damage > Fertile]

Causes of DNA damage:

Intra testicular (Iry testicular) Causes.

- Genodotoxins
- Aging
- ↑ ROS e.g. infection

Extra testicular (External) Causes

- Chemotherapy
- Radiotherapy
- Smoking
- Varicocele

Indications For testing For Sperm DNA damage

1. Unexplained infertility
2. Recurrent pregnancy lss
3. Planning to have IUI

→ So Do "ICSI"

Tests For DNA Damage

↓

Sperm Chromatin Structure assay (SCSA)

↓ upon

- **Nuclear Chromatin decondensation Test**
  - ↓
  - decondensation by some substances as:
    1. Na dodecyl sulfate → lysis of CM
    2. EDTA: → chelate Zinc → decondensation
  - Decondensation ability differs bet fertile & infertile.
- **Aniline Blue staining**
- **Acridine orange Staining**

↑ ↑

- The NL Sperm & NL chromatin will not take the dye (while) the AbNL sperm with disturbed chromatin will take the dye.

Sperm Function Tests

. Introduction:

. Standard (Conventional) semen analysis is not an "accurate" diagnosis or prognosis of Human fertility in vivo or in vitro Except if { Azoo. 100% immotility 100% Necrozoo. } ⇒ are uncommon causes of infertility.

. Most men ē infertility have ↓ Count, ↓ motility or abNL morphology, alone or in Combination (OAT); However, 30% of men have NL x Standard Semen analysis but They are classified as having "Unexplained Infertility"

def . So Sperm function tests aiming at: assessing The ability of sperm to:

1. Penetrate Cx mucus.
2. Traversing ♀ genital tract
3. penetration & Fertilization of oocyte.

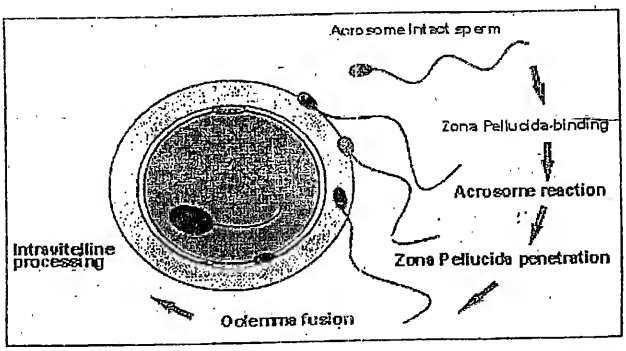

Figure 3-5: Diagrammatic illustration of stages of human fertilization. Spermatozoa swim through the surrounding medium and cumulus mass (not shown) and bind to the surface of the ZP. The acrosome reaction is stimulated by zona proteins and the acrosome reacted sperm penetrates the zona, enters the perivitelline space and binds to the oolemma via the equatorial segment. Oocyte processes surround the sperm head and it enters the ooplasm and decondenses. Infertility could result from defects of any of these processes, e.g. abnormal sperm particularly with defective head morphology bind poorly to the zona.

[1] Sperm - Mucous Interactions (ع إختبارات)

- **In Vivo** (Post Coital test) [PCT]
  - ↓ If -ve or abNL
  - ↓ Repeat
  - ↓ If -ve or AbNL
  - ↓ Invitro tests (Specially Cross over test).
- **In vitro** (SCMC
  - Slide Test (Miller test)
  - Capillary Tub Test (Krem or test)
  - ↓ if -ve
  - ↓ do. "Cross over test"

Invivo Sperm Mucous interaction Teat (Post Coital test)

. **Aim**: to evaluate / Sperm ability of < Penetration & Viability > inside the ovulatory endocervical Mucous e in (9-24 hrs) after Coitus.

Percautions: ① done Exactly at time ("day") of ovulation (detected by:
- BBT
- Serum Estrogen
- Vaginal U/S
- Modified Insler score of Cervical Mucous:

For < ♂ → 4 ds, ♀ → ovulat. time

For Both after Coitus.

تعريف → (2-8 hrs) → ② done within 9-24 hrs after Coitus

③ 2 Samples are obtained by Non Lubricated Speculum from < Vaginal Pool ✓, EndoCervex ✓

④ Abstirence of 4 ds.

NB Insler Score of Cervical Mucous

- Mic exam of Cervical Mucous; Then assessment of:

1. Volume
2. Viscosity (Consistency)
3. Ferning (Crystalization)
4. Spinn barKeit (Elasticity)
5. Cellularity.

↓

Score given for each of these 5 Parameters

- Volume Score:
  - 1 : 0.1 ml
  - 2 : 0.2 "
  - 3 : ≥0.3 "
- Viscosity Score:
  - 0 = Highly Viscous (as in Premenestrual Mucous)
  - 1 = Intermediate
  - 2 = mixed
  - 3 = minimal or Watery (Preovulatory Mucous).
- Ferning (Crystalization):
  - 0 = No crystalization
  - 1 = Atypical "
  - 2 = 1ry & 2ry Stem ferning
  - 3 = 3ry & 4ry " "
- Spinn barKeit (Elasticity):
  - 0 = length of stretched mucous < 1cm
  - 1 = 1–4 Cm
  - 2 = 5–8 "
  - 3 = ≥ 9 cm.

Elasticity: when stretched bet. 2 glass Slides.

: Cellularity:

0 = > 20 WBCs - other cells / HPF
1 = 11 - 20 cells / HPF
2 = 1 - 10 cells / HPF
3 = 0 cells.

Score
- Maximum = 15 ✓
- if < 10 unfavorable mucous
- if > 10 good Mucous.

Interpretation & Significance

- Vaginal Pool Sample:
  - +ve Sperms → successful semen deposition by ♂
  - -ve " → Failed deposition (Coital & / or Mechanical Infertility)
    - d.t < ♂: Deposit disorders (ED, PE)
    - < ♀: Mechanichal disorders (...)
- Endocervical Sample:
  - +ve or NL test → ~~10-20~~ 5-15 progressively motile Sperms / HPF.
  - Poor or abNL Test → < 5 Sperms / HPF (specially ± Circular or sluggish movem).

↑ Viscosity or delayed Liq. ← ♂ (Orig Ejs) → ♀: AbNL Cx mucus.

Causes of poor postcoital test:

a. **Male factors:**
- Improper semen deposition e.g. erectile or ejaculatory dysfunction.
- Poor semen quality e.g. poor motility or count, increased viscosity, or delayed liquefaction.

b. **Female factors:**
- Mechanical causes: Vaginismus, vaginal septum or abnormal cervix e.g. pin-pointed os.
- Abnormal cervical mucus.

c. **Sperm antibodies (immunologic interactions).**

To differentiate between male and female factors in-vitro, Sperm-Cervical Mucus Crossed Invasion test is done.

Examined

NB: inappropriate Timing (1st) the Most Common Cause of AbNL PCT
- its Value → Controversy (NPTs ...)

In vitro tests (اختبارات)

(Sperm Cervical Mucous - Contact test) (SCMCT)

Slide test (Miller kst)

drop of husband's semen (2 in 2 hrs after ejac.)

+

drop of wife's cervical mucous (at time of ovulat°)

↓

put in close (contact) on slide & covered by cover slip

↓

Results

1. NL or good → rapid invasion by large No of Actively motile Sperms ± in (15 min)

2. Poor: Few or No penetration ± d.t either:
   - Abnl Semen (↓ motility)
   - or Abnl Mucous (↑ viscosity)

   "So" → to differentiate "Cross over Test"

3. Abnl test: penetration but either Immotile or Shaking يتذبذب

→ Immunological infertility

Capillary tube (Kremer) test

. See the apparatus.

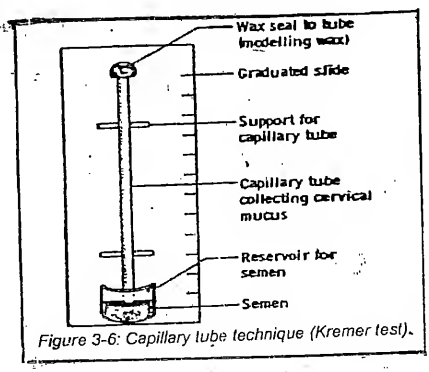

Figure 3-6: Capillary tube technique (Kremer test).

. tube is incubated in a moist environment For 1 hr.

Results the test will be +ve if:

1. No of penetrating Sperms ≥ 50 / HPF
2. distance of penetration ≥ 50 mm / hr.
3. duration of progressive motility should be at least for 24 hrs.

. has Better Pragnostic Value for in Vivo fertil.

. Better > post coital.

Eumarch

Cross oVer Testing [Cross Mucous Hostility Testing]

Aim: if PCT is -Ve or AbNL The Etiology ± abNL Semen or AbNL Cx Mucous to differentiate

Partners Semen tested

&

The Partners Cervical Mucous tested (Ct replaced by bovine Mucous)

- Cervical Mucus of NL fertile Donor
- Partners Cervical Mucous

- Semen of NL Fertile donor
- Partners Semen

NB Indications of PCT:

- unexplained infertility
- High Viscosity
- delayed Liquefaction
- High Vol or low Vol @ NL Count

II. Tests for Assessment of Fertilizing Capacity:

1. HOS Test (& ROS)
2. Sperm Capicitation
3. Zona binding
4. Acrosome Reaction
5. Sperm Penetration

---

1. **HOS test** → see before

2. **Sperm Capicitation:**

- Sperm washing → incubation in Culture media containing Human Albumin → CASA (Assessment)

Hyperactivation Pattern of Motility (Vigrous type of Motility) means:

1. High Curvilinear motility
2. low linearity
3. High amplitude of LHD & Tail displacement

- Results of this:
  Test → Correlated ē preg. rate in ART

3. **Zona Binding assesment (HemiZone assay):**

- Binding of sperms to ZP (1° Zona binding & surrounding ova. Test.) → Acrosome Reaction.
- For assesment of this Binding: "HemiZone Test"

Non living, Non Fertilized Human oocyte is divided into 2 halves:

- ✓ 1 half F+ Capicitated Pt. Sperm
- ✓ 2nd half F+ Capicitated NL Fertile donor sperms.

No of pts bound sperms / Donor bound sperms X 100 = Hemizone Index (if <30%-40% → ICSI (not IUI))

NB 1. Thp test : is an excellent predictor for fertilization rate in ART

2. Cloning of Recombinant Glycoproteins of Zona (Zp3 & Zp2) Recently used will facilitate wide spread of test.

4. Acrosome Reaction assesment:-

detection : 1. E/M (Expensive & Labor intensive)
2. Immunofluorscent staining + HOS Test. (up to)
3. Monoclonal Anti-bodies.
4. Fructose containing in Ags (Recently used as a markers for AR).

x. disadv 1. very expensive
2. Labor intensive
3. Subjective
4. non cost effective
5. represent only 5% of Infertility Causes. (Failed reaction)

. The induction can be done by: by "Ca+ Inophore"

. Assesm b
Spont Induced (in)
pts NL

. In Fertile Men
(SAR) Spont. Reaction rate < 5%
(IAR) Induced Reaction Rate 15-40%

. in Infertile Men
Spont. Reaction Rate is Higher
Induced Reaction Rate is Lower.
(with AR bwps)

. Indications of this test:
detection of Acrosomal defect in pts. ē unexplained poor IVF results so do ICSI

. NB : Acrosomal Index % =

Spermatozoa ē NL Acrosomes (· Size · Form · Staing Capcity)
/ Total No of Spermatozoa × 100

Sperm Penetration Assay (SPA)

(Zona Free Hamster egg penetration test)

Introduction: last step of Sperm-ovum interaction

involves binding of sperms to oocyte mem.

→ Fusion b.t. 2 membs. → penetration of sperm Nucleus into oocyte Cytoplasm.

↓

to do this test:

Hamester eggs (Zona free) (after removal of Zp (C. oopherus) as Zp prevent cross species fertilization)

+

Sperms (of Pt.) (After washing, capacitation induction & suspension in BWW Medium)

↓

- mixing in culture media
- Incubation for 3hrs at 37°C & 5% CO_2
- oocytes: washed → fixed → stained & Examined Mic. for sperm penetration.

- This test requires the sperm to be able to undergo
  (1) Capacitation
  (2) AR
  (3) Fusion
  (4) incorporation to ooplasm.

Interpretation:

- NL test → 10-30% of ovae are penetrated (So can fertilize Human ova)
- Abn'l → < 10% of ovae are penetrated (Infertility) → So IUI or IVI not suitable
- Zero test → Major Impairment of Sperm function Capacity. → Do ICSI

"Exclusion Criteria for IVF" or IUI

disadv: ① Not frontline clinical diagnostic Test & its value is unquestioned.

② don't evaluate Zp penetration.

Indic: ① unexplained infertility to decide IUI/IVF or ICSI

② Pt Candidate for regular IUI or IVF but have Imm. Morphology Screen.

Factors affecting the out come of SPA:

1. No of motile sperms
2. Pattern of Motility: chance of penetration
   ↑↑ with < progressive straight speed (>25 µm/s)
   small amplitude of LHD
   [However NL penetration can occur e⁻ Immotile Cilia Synd]
3. Ability to penetrate Cervical Mucus.
4. Ability to undergo: < Capitation
   AR
   Fusion e⁻
   Vitelline memb.

Semen Components

During ejaculation, these components are not discharged simultaneously, but in a predetermined sequence. Thus, by carefully collecting semen as it is discharged, it is possible to separate out the secretions that make up an ejaculate ("split ejaculate") to make use of the 1st portion in techniques as AIH.

At first, secretions from bulbourethral (Cowper's) and urethral (Littre's) glands, rich in mucoproteins, come forming the prosemen essential to neutralize urethral acidity before ejaculation.

D. Cowpers gland
(2) prostate & testis, epididymis & SV

Semen components	
1. Sperms and secretions from the testes and epididymis	5%
2. Seminal plasma	
a) Prostatic secretions	30% (10-30) (0.5 ml)
b) Seminal vesicles secretions	60% (40-80%)
c) Cowper's glands and Littre's glands	5% (2-5%)

5% < Sperm: Test-sec, epid-sec
5% < Cowper, Littre

Split ejaculate

Volume: Low ... high (60%)

	1st portion	2nd portion
1. Source	Prostate, testes & epididymis	Seminal vesicles
2. Coagulation	Absent (liquefaction)	Present
3. Liquefaction	Rapid	Delayed (20-30 min)
4. Viscosity	Less	Higher
5. PH	Lower (acidic)	Higher (Alk)
6. Sperms	High conc & of better motility	Low conc. & less motility
7. Biochemical products	(A) Prostate (4) PSA: Acid phosphatase, citric acid, zinc, proteases, spermine	Seminal vesicles: Fructose, prostaglandins, protein-like material (for semen coagulation)
	(B) Epididymis: Carnitine, Inositol, Sialic acid, Glycerophosphoryl choline, forward motility protein.	In S.V. secretions may contain suppressive factors → deleterious effects on the sperms.

Clinical uses of split ejaculate → artificial insemination

(1) The 1st portion is used for AIH in cases of high viscosity, large volume, oligozoospermia, or asthenozoospermia. (Polyspermia (Normal No but Large volume)

(2) Tracing the origins of various compounds in seminal plasma e.g. fructose, PG ... etc.

Pro-semen (Cowper, Littre) (5%) → then → First fract.: Testis, Epid., Prost. (5%, 30) → then → 2nd fract.: SV (60%)

Semen Analysis

Basic Introduction

- Semen Analysis <u>Is not</u> a test for Fertility but is a predictive indicator for (functional) status of ♂:
  - reproductive Hormonal Cycle
  - spermatogenesis.
  - patency of genital tract.
- <u>The only True & Sure test</u> for Fertility is Intiation of pregnancy.

<u>Keep in Your mind</u>: that NL values have been difficult To be determined for fertile men during their reproductive period <u>but</u> clinical studies of infertile <u>males</u> have established "<u>Limits of Adequacy</u>"

↓

الى هي عبارة عن مواصفات السائل المنوي الطبيعي (<u>NL Semen</u>) الى تحتها او اقل منها يعتبر <u>الحمل</u> (زوجة) صعبة وليس مستحيل"

(So subfertile Not infertile Except if AZoo. → infertile or sterile).

- <u>So these limits are not (absolute) why??</u>
  1. Some fertile male have semen quality <u>below</u> These limits & some infertile males having semen quality within NL Limits of Adequacy.
  2. <u>Standard</u> Semen analysis doesn't asses the Functional <u>integrity</u> of sperm (Standard analysis may be NL <u>but</u> sperm function tests may be AbNL → Failure of conception)

"مهم" <u>NB</u>: 30% of men ē Adequate Standard Semen analysis → Having abNL sperm function. (Hence they are: infertile)

Semen Evaluation
(Semen Analysis)

- **Standard Semen Analysis** (Conventional)
  - ١. كيفية تجميع العينة
  - ٢. Physical Exam
  - ٣. MiC Exam
- **Advanced Semen Analysis**
  - **Optional Tests**
  - **Sperm Function Tests**

18 Points ... هاذ فقط

Standard Semen Analysis
(Limits of Adequacy)

A. Sample Collection

1. Abstinence period: (الامتناع)

- 2-7 days (Typically 3-4 ds)
- if < 2 → oligozoo. &oligospermia
- if > 7 → polyasthenozoo.
- Abstinence is the major source of variation in Semen parameters [sperm Count ↑↑ 25% / day]

2. Perform 2 Analysis:

- لازم المريض يكون معاه ٢ كيلوس من مكانين مختلفين
(2 analyses 1-3 ws apart) → (أو أكتر 2-4 ws)

3NB
- Why doing 2 analyses? because men have great variations in their Semen parameters.
- If there is great Variation bet. the 2 → (do 3rd Semen Analysis)
- ؟ ما هي الحالات اللي ممكن نعيد فيها التحليل بعد فترة ٣ شهور
  ↓ if.
  1. Exposure to high fever or illness
  2. Exposure to Cytotoxic agent or drugs.

3. Collection Method:
- Should be at Lab but not at home Collect (by Coitus interruptus) (but Transport it in 1-2 hrs)
- obtain it by: Masturbation (should be dry) but non spermicidal Vaginal lubricants may be used (as Vasiline or Olive Oil)
- Avoid Condom: Contain spermicidal substan
- Coitus interruptus ??
  - presemen may Contains Spermatozoa ??
  - 1st fraction of ejaculate Contains very much spermatozoa
  - Contaminate by Vaginal acidity discret.

NB
Special Condoms Now used called "Collection Condoms": made of silicon or poly urethan (as Latex is spermicidal).

4. Container & Temp
- Clean (plastic or glass) not Toxic to spermatozoa.
- 20 - 40 °C (room - body Te)

5 Period before Exam: (عزلا على العلى ان حريد ترفع الحصة وكذلك لتقل سرايمك للعمل)

- If the previous semen was NL, Period → (within) 1 hr.
- If the previous semen was abNL → < 1 hr
- If Microbiology is needed → ē in 3 hrs.

⑥ Culture: If culture is needed; the patient should pass urine then wash his hands & penis before ejaculation & microbiology should be performed ē in (3 hrs)

NB: split ejaculate collection may be needed.

Ⓑ. physical Examination

1. Color
2. odour
3. Volume
4. Liquefaction
5. Viscosity
6. PH

1. Color:

- NL → grayish opalescent
- AbNL:
  - whitish → Excess No. of Sperms or WBCs.
  - greenish → Genital inf.
  - Yellowish → 1. Long abstinence
    2. Urine contamination → BN drug.
    3. Bilirubin → LCF
    4. Yellow + clumbs + bad odour → inf.
  - Red → 1. Drugs → Rifampacin
    2. Blood (Hemospermia):
       - pink → Fresh Traces
       - Bright red → Fresh Excess
       - Brown → old Excess.

2. odour: Chic odour is d/t Spermine of Prostate.

3. Volume:

- NL: 2–6 ml (≥ 1.5 ml)
- Abnormalities may be:

Spermia = ↓ seminal fluid

2a Spermia = sperm

* Aspermia (Absent semenal fluid) = Anejaculation (قذف)

* Low volume (< 2ml)
  - Oligospermia
  - Hypospermia
  - Paucispermia
  - Causes:
    1. short abstinence period
    2. Faulty collection
    3. Accessory gland dysf. or Atrophy.
    4. RGE, retrograde
    5. ejac. duct obst. (unilat.)
    6. Androgen deficiency → Accessory glands Atrophy. → Seminal vesicle disorders
  - Chr. TB or Bilharziasis → fibrosis

* Excess vol (> 6ml) (Hyperspermia) Polyspermia
  - Causes:
    - long Abstinence period
    - Contamination by urine
    - Acute inf.
  - Effects:
    1. Relative Oligozoo.
    2. loss of Large part of semen after intercourse.

4. Liquefaction:

Semen after ejaculation has 3 Forms:

Liquid → (immediately) Coagulated → (20–30 min, "15 min", (< 30 min)) Liquefied.

- Coagulated: from SV: dil. of Proteins (as Kinase or Seminogelin)
  - Failed in: SV disorders
    - CBAV
    - ej. duct obst. (EDO)
- Liquefied: from Prostate d.t their Liquefying enzs.: (Proteases = Fibrinolysins)
  - PSA
  - PAP
  - PBP (from Cowpers)
  - Tissue plasminogen Activator
  - Failed or delayed Liquefaction (> 30 min) → prostatic disorders e.g. Prostatitis.

appears: "Heterogeneous gelatinous clots inside Liquid"

Semen should be liquefied in 20–30 min
If > 30 → delayed Liquef.

- Liquefaction & infertility
  - To diagnose delayed Liquefaction as a cause of infertility. Sperms should be absent in post Coital test.

- Treatment of delayed Liquef. (>30 min) → AIH

After Exclusion of Prostatic disorder → Add Liquefying enzs (0.2% Amylase, α-chemotrypsin, Hyaluronidase) → Then AIH

5. Viscosity

- def: resistance of the fluid (semen) to flow or its "Thickness"
- Estimation: By allowing the completely Liquefied (30 min) Semen To be poured Through wide (bored) 5mm pepitte

NL Viscosity (Normoviscous)

- Semen Comes as discrete drops (drops by drop) from The Pepittor < 2Cm thread.
- Grading: From:
  - 0: consistency of water (To)
  - 4+: " " gel.

High viscosity

- Semen Comes in Threads > 2 Cm from The pepitti.
  or
- quantified by measuring The time needed for one drop to leave The pepitte. =

Etiology: SV disorders (evidence ↓ Fructose and acid, Hyperviscous Semen)

Note that

. Viscosity & infertility:

. role of ↑↑ Viscosity in infertility is Controversy as many patients with Viscous Semen Can achieve Conception.

(So) . don't Consider Viscosity as a Cause of infertility Except if there is:

① Persistent high Viscosity دائما بتكون عالية

② Absent or Very low No of sperms in post Coital test (PCT)

(↓ motility, ↑ chromatin stability) ← repl. . High Viscosity may lead to (effect on semen):

①. Easy drainage after intercourse خارج cervix

②. Failure of Coating The Cervix

③. ↓ motility → inability to enter Cervical Canal.

④ ass. c High chromatin Stability.

(N B) to differentiate bet. High Viscosity & delayed or Failed Liquefaction:

- Failed Liquefaction: Heterogenous gel masses in more fluid base.
- High Viscosity: ↑ thickness & Come in Thread > 2cm from the 5 mm bored Pipitte

. Treatment of High Viscous Semen: → AIH

- Addition of Enzymes (as in Liquef.)
- " of Culture media. (... التخفيف)
- Repeated Needling: repeated passing of semen Through "19 G Syringe" (avoid it as it → deterioration of semen motility).

then → (AIH)

if you see < delayed Liquefaction: / or / High Viscosity. > → Repeat Semen analysis & Exclude < prostah / SV / causes

If persistant ← Post Coital test ← No Viscosity ← sperm in cervical mucosa / or not aware of infertility

6. PH of Semen:

- NL: 7.2 - 8.8 (Alkaline) [d.t bicarbonate] → antagonize Vaginal Acidity.
- Note that:
  - Prostatic sec. → slight acidic (6.5)
  - SV sec. → Alkaline.
- So: Alkalinity of Semen is d.f SV Secretion.

- Abnl PH:
  - < 7 + Azoo. → CBAV
  - > 8 → Genital infection.
  - SV obstruction / Hypofunction

NB: Abnormalities of SV & prostate: may alter the PH.

SV (Volume, Coagulat°, Viscosity, PH) → So in CBAV or ejact. duct obst:

- ↓ Vol (Hypospermia)
- Failed Coagulat°
- ↑ Viscosity
- ↓ PH (Acidity)
- Azoospermia.

if you see < delayed Liquefaction / or / High Viscosity > → Repeat semen analysis & Exclude < prostah / SV causes

If persistant → Post Coital test ← No viscosity ← sperm or not acquire of infertility

Post coital cervical Mucosa

6. PH of Semen:

- NL: 7.2 - 7.8 or 8 (Alkaline) [d.t bicarbonate] → antagonize Vaginal Acidity.

seminal ves. secretion

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- ↑ Viscosity
- ↓ PH (Acidity)
- Azoospermia.

"Microscopic Examination of Semen" (6)

1. Concentration (Count)
2. Motility
3. Viability
4. Morphology
5. Agglutination
6. Non Sperm Cells

---

1. Sperm Concentration:

لازم تراعي هاتين القيمتين معاً

- Sperm Conc: 1ml : should be > 20 million/ml (20 - 200 - 250)
- Sperm Conc/ejaculate (Total Count/ejaculate) : should be > 50 million.

هاتين القيمتين لازما تكون الأصحية بدليل :

- If Sperm Count/ml = 15 million/ml $\xrightarrow{So}$ "AbNL"

& If ejaculate vol. is 5 ml, So total count/ejaculate will be 5 ml X 15 mill. = 75 milion $\xrightarrow{So}$ "NL"

So the 2 values should be at NL to fulfill the NL semen analysis.

- Abnormalities in Count may be:

1. AZoospermia → complete absence of spermatozoa (Testis, transport → No Azo)

"قاعدة" AZoospermia Not Considered Except after Repeated Centrifugation of Semen

2. Hidden (مختفي) Cryptozoospermia: → If the semen sample is Azoospermic → Repeated Centrifugation, If there are sperms in the Sediment called "Cryptozoospermia" If No sperms detected → Azoospermia.

- NB: (Cryptozoo = Hidden Spermatozoa = apparently Absent Spermatozoa)

Oligo Zoospermia (Count < 20 million / mp or < 50 mill. / ejaculat)

Causes

False

1. Short Abstinence period
2. psychogenic Incomplete ejaculation (anxiety)
3. Loss of 1st part of ejaculation (faulty collection).
4. Hyperspermia: large vol. of seminal plasma → relative oligozoo.

True (persistent oligozoo.)

1. Idiopathic: Commonest cause.
2. Genetic:
   - AR inheritance usually present
   - usually ass. ē severe oligo (< 5m/ml)
3. Obstructive: Partial or unilat. ejac. duct obst.
4. Impairment of Spermatogenes
   - Varicocele.
   - Infection
   - Drugs
   - fever
   - systemic illness.
   - smoking
   - orchidopexy
   - Antisperm antibodies
5. Isolated FSH deficiency.

NB: Severe oligo zoosp. (< 5 million/mp) ↓ do

- A. Genetic Screening:
  - Karyotyping (if < 10 millions)
  - Yq micro deletions (if < 5)
  - CFTR = cystic fibrosis Transmembrane Regulator.
- B. Genetic Counseling & Hormonal profile

(Specially if ICSI is indicated)

Treatment of Oligo Zoo

- Treatment of Treatable Causes.
- Non treatable causes → ICSI

NB: Hormonal th now considered of No Value (Except if FSH deficiency) as
- Gonadotropin inj.
- Clomiphene
- Tamoxifen
- Testosterone.

4 Polyzoospermia: (Count < 200 or 250 million / ml)

- AEI [False: physiological polyasthenozoosp. d/t Long abstinence period.
  True: persistent.

- Role in infertility: Controversy but ± ass. with:

(1) ↓ Fructose (Consumption by large No) → ↓ motility

(2) Spontaneous Abortion in wives

3- Variable results of Hamster egg penetration test.

(1) repeated analysis. (↑ excluded false..)
(2) Repeated ejaculation in vitro stim. of motility & AIH.

NB: Methods of Sperm Count

1. Rough method
2. Hemocytometer
3. Makler Counting Chamber
4. Electronic Counter
5. DNA Flow Cytometry

1 Rough method: (No / HPF x 10 6):

- Counting the mean No of sperms in several fields under a (40x) objective & multiplying x 10 6
- eg 60 sperm / HPF → (roughly / i.e.) 60 million / ml
- Should be done during intial Exam. to determine the dilution needed during hemocytometer Counting.

2. Hemocytometer:

- The most accurate method
- عبارة عن شريحة مقسمة الى مربعات كبيرة (1mm²) كل مربع كبير مقسم الى ١٦ مربع صغير بينهما ٥ سم مربعات إضافية لتحديد المربع الكبير اللي جنتر الشريحة وعدّ الحيوانات المنوية

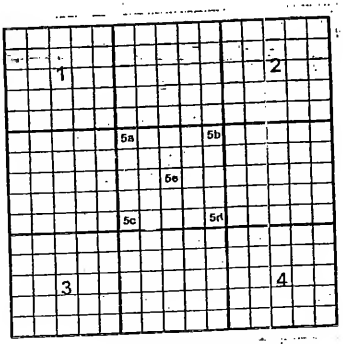
- at first before Counting:

1. Spermatozoa should be immobilized

1:20 → if Sperm Count (by rough method) is > 50 /HPF

1:10 → if sperm count (N N H) is < 10 /HPF

NB
Only recognized Spermatozoa are counted not the Free Sperm tails



(HemoCytometer)

3. MaKler Counting Chamber :

- Special Counting Chamber for rapid Seman analysis have became popular & gained the widest recognition & use.
- No dilution is used So, No of Sperms Counted / 10 Squares = Sperm Conc. in millions / ml.

(Causes Errors) → . Not recommended for routine use & when WHO Standards are desired but used as a simple method for less Specialized Labs.

4. Electronic Counter: Easy but expensive.

5. DNA Flowcyto
. rapid & precise
. haploid sperm differ in Staining ch than othe diploid cells in Sem

2. Sperm motility

1. at least 200 spermatozoa should be screened
2. should be immediately after ejaculation & $\leq 1hr^{-2}$ (30-60) after that (preferably by 30 mins after ejac).
3. Should be both — Quantitative ✓ نسبة الحيوانات المتحركة. — Qualitative ✓ كيف تتحرك.

for assessment.

Should be Assured

by

- 30-60 mins after ejac.
- 37°C

(EAU)

I. Quantitative Assessment

Counting the motile & immotile in at least (5) fields.

$$\% \text{ of motile} = \frac{\text{No of motile}}{\text{Total No of sperms}} \times 100$$

(WHO 1999) NLly: at least 50% should be motile. (≥50%) show progressive motility.

II. Qualitative assessment

Subjective Methods

I. Acc. to degree of Forward Progression: ✗

- 0 : None: non motile
- 1 : Poor: Weak forward Progressive
- 2 : Mod: definite " "
- 3 : Good: Good " "
- 4 : Excellent: Vigrous " "

II. Acc. To WHO 1999: ✓

- Grade a : Rapid progressive (≥ 25 μm/S)
- " b : Slow (sluggish) " (5-25 μ/~)
- " c : Non progressive (< 5 μm/S)
- " d : Immotile

"Flagellar activity."

Objective Methods (بدون تدخل) CASA

1. Prolonged time Exposure photomicrography: photograph show Tracks produced by sperm head across the field during 2 sec. exposure
2. Multiple Exposure photography: successive photographs of sperm along the field & from change the position; velocity can be calculated.
3. Video micrography.
4. Laser light.
5. CASA.

Normally: ≥ 50% should be progressively motile

شرطين: ≥ 50% / progressive (good)

or

· Acc. To WHO { Ga ≥ 25% (or) / Ga+b ≥ 50%

احفظ كويس

أي واحد تحقق من شرط الاثنين

Asthenospermia: if [Ga < 25% or / Ga+b < 50%

.. Causes ?? See infertility
· inf.
· Varicocele

So: Motility if:

- NL → Means:
  - ≥ 50% progressively motile
  - Ga ≥ 25% or Ga+b ≥ 50 (WHO)
- AbNL (≥ 40% immotile) → Viability test
  - Necrospermia
  - Asthenospermia → do E/M

Immotile or Severe Asth. < 5-10

Severe Asth. < 5%

< 40%

NB

→ (a) = Progressive Motility → Strong swim fast in straight line

→ (b) Non-linear " → move forward but in curved or crooked mot

Motility disorders

→ > 50 should p— pro—

GA > 25%

Gb → 10%

Ga+b → 75%

3. Sperm Viability (Vitality) ... Asthenozoosp.

. def. Immotile sperms may be < living or dead (→ Necrozoospermia) to differentiate bet. them do these tests..

. Indications:

1. Very low No of sperms
2. if > 40% of sperms are immotile graded d to differentiate bet immotile living (Asth.) & immotile dead (Necrozoosp).

(very low or absent Mot. < 1% (5-10%))

. Idea: depends on the Cell membrane status:

. Intact → in living sperm's (so don't take the dye)
. damaged → in dead sperms (Take the dye).

. Tests for Vitality:

1. Dye Exclusion method (N/E Stain)
2. Hos test
3. Invitro stimulation.

طريقة أولى

. 1 drop → Semen (100 spermatozoa)
. 2 drops → Eosin Y 1%
. 3 drops → Negrosine 10%

→ all mixed on glass slide Then a drop be put on another slide & Examined under phase contrast Mic.

- 0.5 g EY
- 3 g N
- 30 ml distilled water

N/E sol
2 drops
Semen drop.

Result <
- living sperms (intact memb): will not take the dye → appe White
- dead sperms (damaged memb): will take the dye & appear as red (d.t E) against the Violet background (d.t Neg).

2. "HOS" test (Hypoosmotic Swelling): Hypoosmotic Solution (150 mmol/L) + Spermatozoa →

Result { Living sperms: (Semipermeable membrane maintain osmotic equilib.) → (Swelling) & plasma membrane & Curling of tail.
dead sperms: (leaking membrane) → No swelling (retain its morphology)

③ Invitro stimulation of Motility: by using Kallikrein or Caffeine.

NB. * These tests used during ICSI to select the Immotile But Living sperms.

* in Cases of Necrozoo → Obtain sperms from testis (Viable.)

* Apoptosis: an important mechanism of NL Spermatogenesis & its disturbance impaired quality.

Staining of Apoptotic sperms by:
- Staining
- Flow Cytometry
- Terminal deoxy Nucleotide mediated dUTP nick end Labelling (TUNEL).

% of Apoptotic Sperms (0.1% - 5%)

Normal Viability: Sperm Vitality or Viability should be ≥ 75%
→ (>75% of sperms should be living)

Necrozoospermia ← لو أقل

4. Sperm Morphology

1. 2 Criteria (For assessm % of NL) → WHO / KSCM (Kruger strict Criteria of Morphology)
2. 2 MiC Exam → LM / EIM
3. 3 Indices → TZI / MOI , SDI (TZI > Roy's Acrosomal index.)
4. 3 Anomalies → OAT / GloboZoo58. / Sterelizing Sperm defect.

① 2 Criteria used for Assesment Morphology : (100 Spermatozoa evaluated). or (better 200)

WHO

NLLy (NL Semen) : NL Forms should be ≥ 30%
& AbNL " should be < 70%

- So Teratozoo Spermia Means:
  NL Forms are < 30% → by WHO
  < 14% → KSCM

KSCM (الأكثر استخداماً)

- depends on: Counting The Border line Sperms as a bNL (WHO Border line → NL) So Called "Strict"
- if > 14% of Sperms are of NL morphology → NL Semen
- Reference of NL Sperms: sperms from endocx & Zona binding.

② 2 methods of Morphology assesment:

Ⓐ LM → after staining with:
- H & E
- Eosin
- Giemsa
- Papanicolaou (الأفضل والأهم) (تلطيخ بابانيكولاو)
- Leishman stain
- Shorr.

WHO Recommend: pap. · shorr · Diffquick stain.

Ⓑ EIM → to diagnose ultrastructure defects. e.g Axonemal defects.

- See Criteria of NL & Varieties of AbNormal Sperms

(2nd) Higher predictive Value for determining rates of pregnancy in IVF
if KSCM > 14% → 91% FR
if KSCM < 14% → 37% FR.

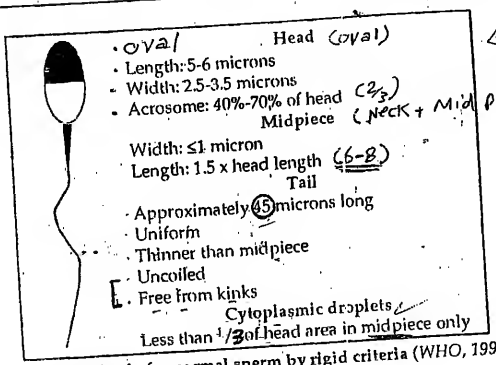


Fig. (24): Criteria for normal sperm by rigid criteria (WHO, 1999).

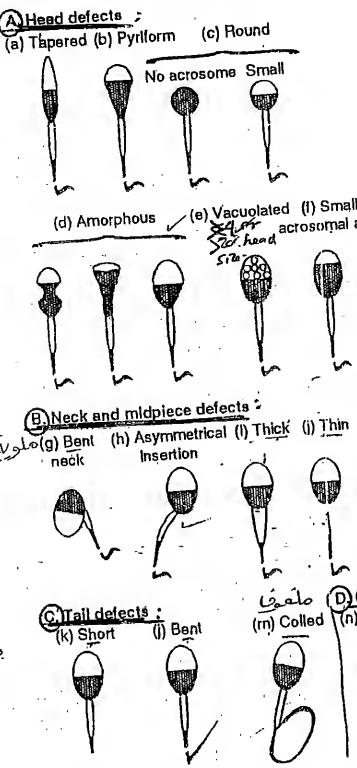


Fig. (25): Schematic drawings of some abnormal forms of human spermatozoa (WHO, 1999).

4 Sperm Agglutination & Aggregation.

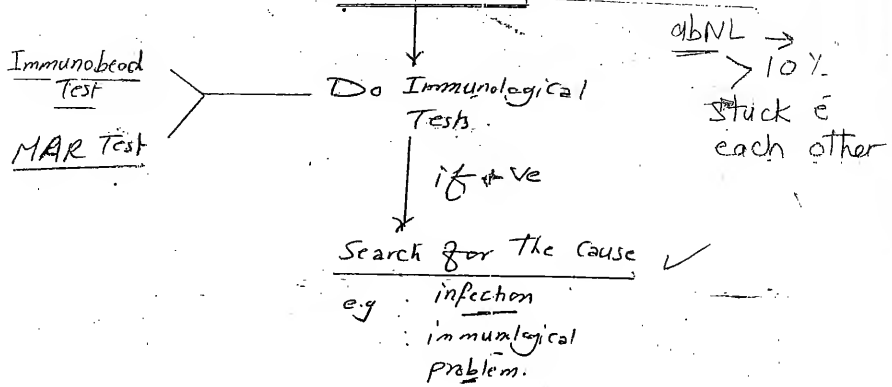
- **Sperm Agglutination** (if > 10% → significant): living, motile spermatozoa stuck to each other by antibodies with minimal involvement of other cells &/or debris.

- **Sperm Aggregation**: dead sperms stuck to other cells &/or debris rather than to each others.

N.B 1. at least 10 fields should be examined ✗

2. appears:
   - clinically: small clumps in NL semen
   - M.C. stuck may be ⌜ Head to Head, Head to tail, tail to tail.

3. Agglutination is significant if > 10% of sperms are agglutinated.



NB: (1) Any borderline sperm → Counted as AbNL

(2) Teratozoospermia means:

- Normal forms < 30% WHO or < 14% KSCM
- TZI is > 1.5.

2 Indices → T.Z.I (MAI)

(Teratozoospermia index = Multiple anomalies index)

= Total No of defects / No of AbNL forms

= No of defects / Each sperm ✓

If 1 means: each AbNL sperm has one defect.

If 3 means: Each AbNL sperm has 3 defects.

NLLY: TZI < 1.5 & if → good prognosis

> 1.5 → Significant ↓ in fertility.

& X MoI (Motile Oval index)

Used to evaluate the 3 sperm parameters at the same time:

= Count x % of motility x % of oval sperms

So lower limits of MoI =

20 x 40% x 60% = 4.8 million sp. Imp.

Also SDI (sperm deformity index) = No of defects / Total No of sperms

NLLY: upto 1.5

3 Anomalies

OAT (oligoasthenoteratozoosp.)

dg: ↓↓ of 3 sperm Parameters:

- Count
- Motility
- Morphology

[Stress Pattern]

occurs in any condition Ass c Testicular Stress i.p. dg

- Varicocele
- Heat Exposure
- Systemic illness.

[- Fever
- irradiation.

Cryptic →

Globozoospermia (Round head Synd)

* Sperms that show:

- By LM: Round Head
- By EM: defective Acrosome (Absent or small). < 2/3 head

Sterelizing sperm defects

= Globozoospermia + Immotile Cilia Synd. (Kartagener's)

Immunological tests include

Immuno bead test

Poly ceramide beads Coated ē Anti human Ig's (IgG / IgA)

+

Suspension of (Sperms) (200)

(Centrifugation → remove seminal plasma)

↓

Comment on: ① % of Sperms bind to beads
② pattern of Binding
③ Class of Abs.

Test is +ve if ≥ 50% of Spermatozoa bind to 1 Immuno bead → Immunologically infertile.

Mixed Antiglobulin Reaction (MAR) test.

→ Latex Coated with anti Human (or Sheep RBCs) Igs (IgG / IgA)

+

Fresh Semen Sample (No Centrifugation)

↓

+ve test indicated by demonstration of Sperms attached to the Latex.

The test ±:

- −ve: if < 10% of Sperms attached to the Latex
- +ve: if 10–90% of Sperms attached to 1 Latex
- Stay +ve: > 90% ...

NB: the most specific is Immuno bead test.

- others tests
  - RIA
  - Sperm Immobilization Test

[6] Non Sperm Cells. (Round Cells)

Def → Cells other than sperms that are present in semen → Collectively Known as Non Sperm Cells or Round Cells.:

أهم حاجتين دول اللي هنحفظهم →
- ✓ Leukocytes
- ✓ Immature spermatogenic Cells.
- Epithelial Cells
- ✓ RBCs
- protozoa

→ To differentiate bet them

WBCs

All semen samples Contain (WBCs)

- NL level < 1 million / ml or < 5 / HPF (high power field)
- Excessive No → infection (Leukocytospermia = Leukospermia) → ↓ sperm quality (by ↑ ROS)

Con be detected by:

[1.] Peroxidase test: depends on the presence of peroxidase Enz. in granules of PNL

disadv: Can't Identify:

[a] Activated WBCs ū released their grs.

[b] WBCs: that don't Contain Peroxidase.

[2] Immunological method: using monoclonal Antibody to detect Leukocytes specific Ag (CD45). [Leukocyte pan markers]

Immature Spermatogenic Cells

- include:
  - Immature Spermatid.
  - Spermatocytes.
  - Spermatogonia.
- Significance:

  Their Excessive presence indicate → defective spermatogenesis (shedding)
- Stain:
  - Leishman
  - PAS (for Acrosome

Functions of The sperms

- Capicitation
- Acrosome Reaction

Inside Male Genital Tract

1. Sperm Motility
2. Fertilizing ability

Inside Female Genital Tract

1. Inside ♀ Genital Tract (Uterus, Tubes)
2. Sperm ovum interaction

I. Function inside ♂ genital Tract

Sperm motility

1. Non motile testicular sperms are transformed into motile sperms inside the epididymis
2. Energy required for motility is derived from Mitochondrial ATP

(ATP $\xrightarrow[\text{enz.}]{\text{ATPase}}$ ADP $\longrightarrow$ energy release)

- Mechanism of Motility:
  - ATPase enz. is Activated inside one half of the Axonemal ring $\longrightarrow$ The dynein arms will pull on the adjacent doublet microtubules of this side $\longrightarrow$ Sliding of doublet microtubules $\longrightarrow$ bending of tail in specific direction $\longrightarrow$ switch of the process

Sperm Fertilizing Ability

- Testicular sperms have No Fertilizing ability but attain it inside the epididymis.

Activation
sliding
bending
of to other side of the Axoneme → bending of Tail to the other direction.

Detailed Mechanism of

- Dynein: is Mg dependant ATPase → driving force for motility.
- Doublet Microtubs. interact each other → sliding &
  - Central
  - Nexin
  - Spoke: → interact & Convert the tubular sliding To "Flagellar Wave form"
- 4 elements responsible for Initiation & maintenance of Motility:
  - cAMP
  - Ca+
  - protein phosphorylation
  - protein Kinase A (PKA) (key enzyme).

  Both PKA & protein Phosphorylation is cAMP dependa
- Other factors that help (in semen):
  - Zinc
  - Citric acid
  - Fructose
  - bicarbonate
  - PGs
  - Choline Compounds.
- The fibrous sheath of the tail is composed of Flagellar protein called outer dense fiber protein (ODF) which is passive-elastic & rather stiff element that Transmit the Kinetic Energy from the axoneme to words the junction of Flagellum & Sperm Head. & d.t bigger diameter at this point → Torque.
- Zinc: Concentrated in Large amount (75%) to ODF. "Sperm"
- Role of ROS: in Normal events: regulate Capacitation AR → Controlled by balance between it & Scavenger
  - if ↑↑ → oxidative stress
    - effects: 1. Lipid peroxidation 2. axonemal damage.
    - Source: NL & dysfunctional Sperms, WBCs
    - main Scavenger: SOD & GPX others (Vit C, Zinc, Vit E)

II. Function of the Sperm inside the Female Genital Tract.

1. inside < Cx / UT — < penetration of Cx Mucus / Migration through the UT.

س: ماذا يحدث؟

2. inside the tubes < Sperm Capicitation & Hyperactivation.

س: لماذا يحدث؟

3. Sperm ovum interaction.

س: متى يحدث؟

So Sperm function inside the ♀ Genital Tract

عبارة عن رحلة الحيوان المنوي داخل "female genital tract"

وهذه الرحلة عبارة عن ٣ خطوة.

((Steps))

usually: 200 – 600 million Sperms are deposited in Post. Vaginal Fornix.

1. Rapid Transport

- immediately after ejaculation
- Takes: 10 – 20 mins.
- rapid Transport of sperms inside the cervical canal helped by UT. Contraction during Coitus. (AF < motility + UT Contract)

"To be protected from vaginal acidity"

↓

2. Sperm Colonization & reservior Formation

- Takes : upt 48 hrs.
- Colonization of Large No Inside the Cervical Crypts → Formation of sperm reservior. (ensures : Constant release & ↑chance of Fertilizat°).

3. Slow Release & Transport :

- Sequential release of Sperms from The cervical reservior helped by UT. Contractions d.t PGs inside The Semen (secreted by SV).

4. Sperm Capicitation : → في UT Tubes

- decapicitating factors: Acquired from epididymis & SV to prevent "premature Capicitat°."
- "NO" may play a role. (في الدم)

def: Changes that occur for the sperms after separation from the Seminal plasma (الي عادة contain decapicitating factors that prevent Fertilizat°) during Transport through the UT & Tubes.. so it will be Capable of Fertilization.

- requires 3-4 hrs. during الـ Sperm

- الـ memb. shows the following Changes:
  1. removal of Glyco Protein Coat & Semin. plasma proteins from the plasma memb. over / acrosom
  2. Destabilizaton & ↑ Permeability.
  3. Efflux of cholesterol.
  4. Influx of : Na^+, K^+, Ca^{++}, O_2 & Glucose.

5. Sperm Hyper activation

(في الجنين)

3 Changes:
- Vigrous ↑ in amplitude of tail bending.
- ↓ Linear (Progressive motility)
- attainment of Circular motility.

↓

6 & 7. Penetration of

- Cumulus Cophorus : depend on Hyaluronidase enz. (Hyaluronidase enz.)
- Corona Radiata : depend on esterase enz. (Corona penetrating enz.)

↓

8. Zona binding : d.t interaction bet. specific sperm receptors & specific ZP protein (ZP3)

↓

9. Acrosome Reaction (AR) (Zona Penetration).

def. : process of Activation of proacrosin to Acrosin followed by Zona Penetration.

- Mechanism :
  - The Acrosomal CAP has 2 Layers :
    - outer Acrosomal memb (OAM)
    - Inner Acrosomal memb. (IAM)
  - Contain : inactivated enz called Proacrosin.
  - Steps of Activation :
    1. lifting or elevation of plasma memb. → outer acrosomal Membrane
    2. dispersion of OAM
    3. Formation of multiple fusion pairs bet. 2 memb. → Fusion Pores
    4. dispersion of Fused membs. as a vesicles
    5. Exposure of IAM → Proacrosin → Acrosin → Zona Penetration ✓

ZP : penetrated by Acrosin enz.

↓

10. Zona penetration

↓

11. Fusion bet. Sperm membrane & oocyte Membrane

↓

12. Cortical Reaction (Zona reaction).

exocytosis of Cortical granules → Cortical reaction → Hardening of Zona → polyspermy Blocking (-- of Zona penetration by other Sperms).

↓

13. Formation of Male Pronucleus [Chromatin decondensation]

↓

14 Nuclear Fusion : DNA of sperm + DNA of ovum → Zygote.

NB

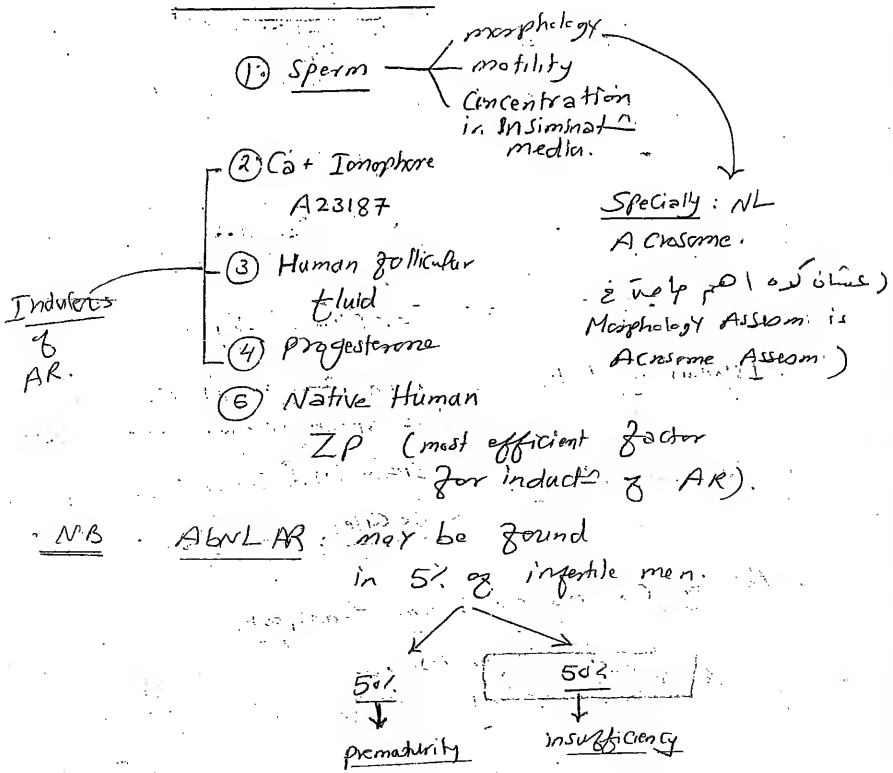
- The sperm pass by movements of their tails through the Cx. Canal but passage through the UT & Tube is assisted by muscular contractions of them.

So: at cervix → sperm motility ... after Cx:
- Sperm motility
- Myometrial Contract^
- Mesalpinx "
- Female orgasm.

- Isthmus of F. tube: is main functional reservior where sperm remains until ovulation.
- Survival time for: Sperm inside female Tract: upto 48 hr.; Oocyte: 12-24 hrs.
- Role of Cx during sperm transport:
  1. Sperm reservior
  2. Leukocytic reaction: Cx will ↑↑ No of WBCs in mucous → present ig.
  3. mid Cycle Mucus → allow sperm Penetrat^
  4. Immune Suppressive role.
  5. energy to the sperm
  6. Sperm select^: only motile & NL Can Penetrate (see KSCM)

- For Staining of Capicilation & Acrosome React^ → "Chlortetracycline Staining"

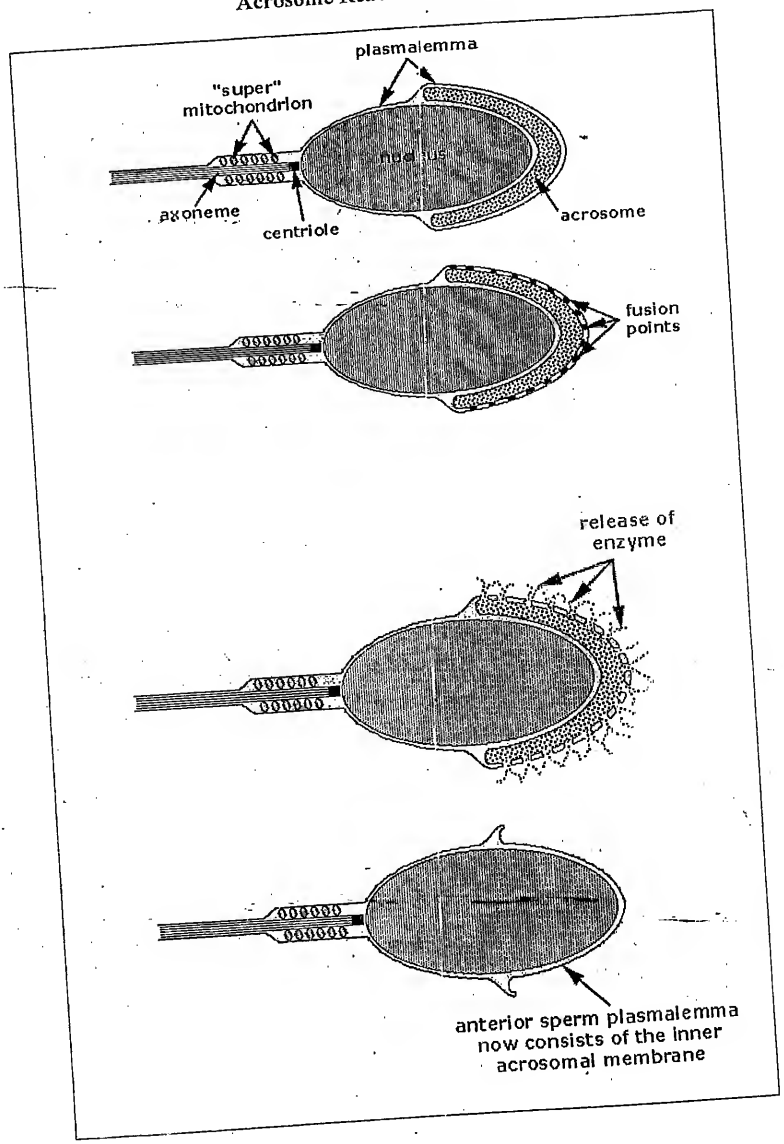
- Factors affecting Zona binding & Acrosome reaction



- NB. Abnl AR: may be found in 5% of infertile men.

- Pentoxifylline: doesn't ++ AR but ↑ sensitivity of Sperm to Ca+A23178. Ionophore.

Acrosome Reaction



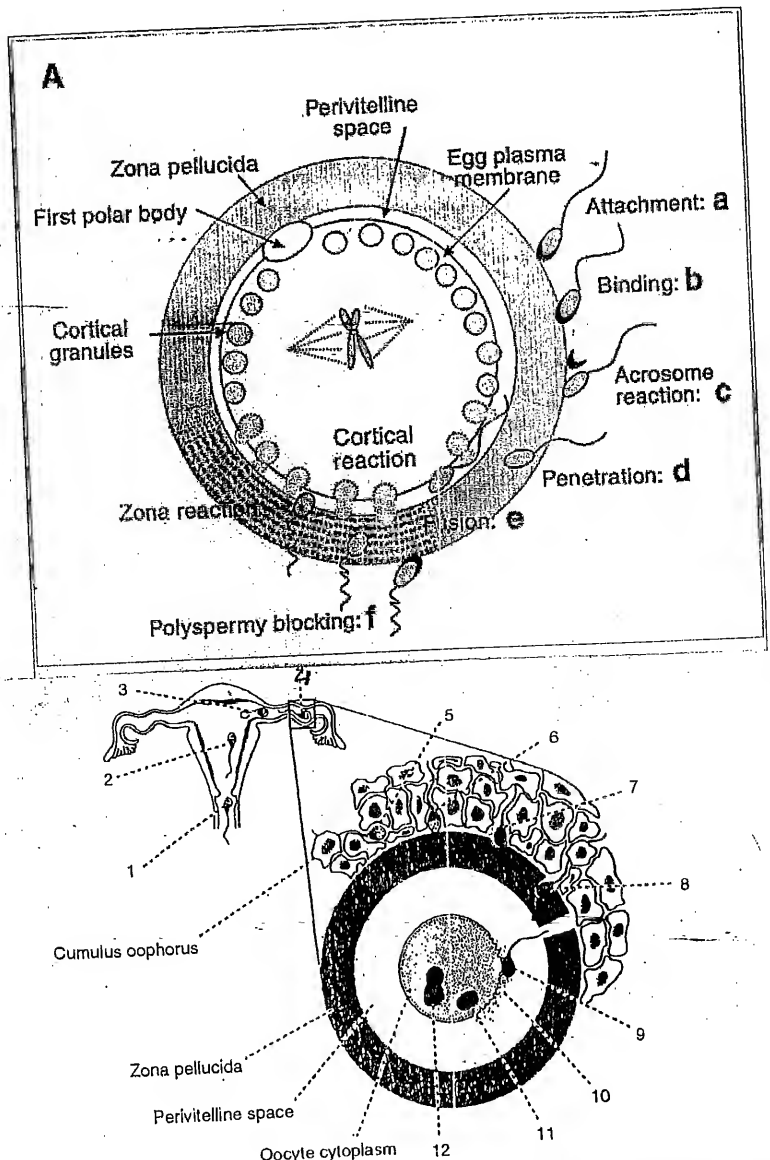
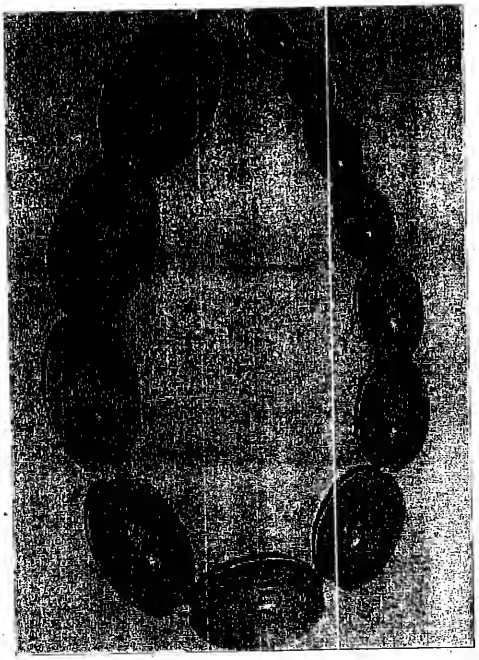
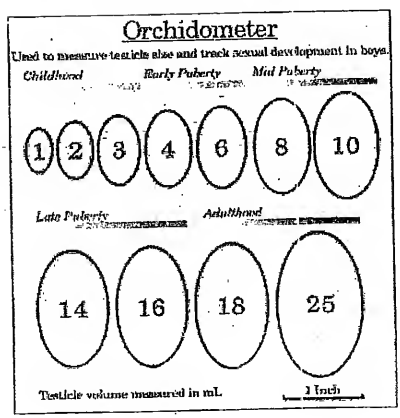
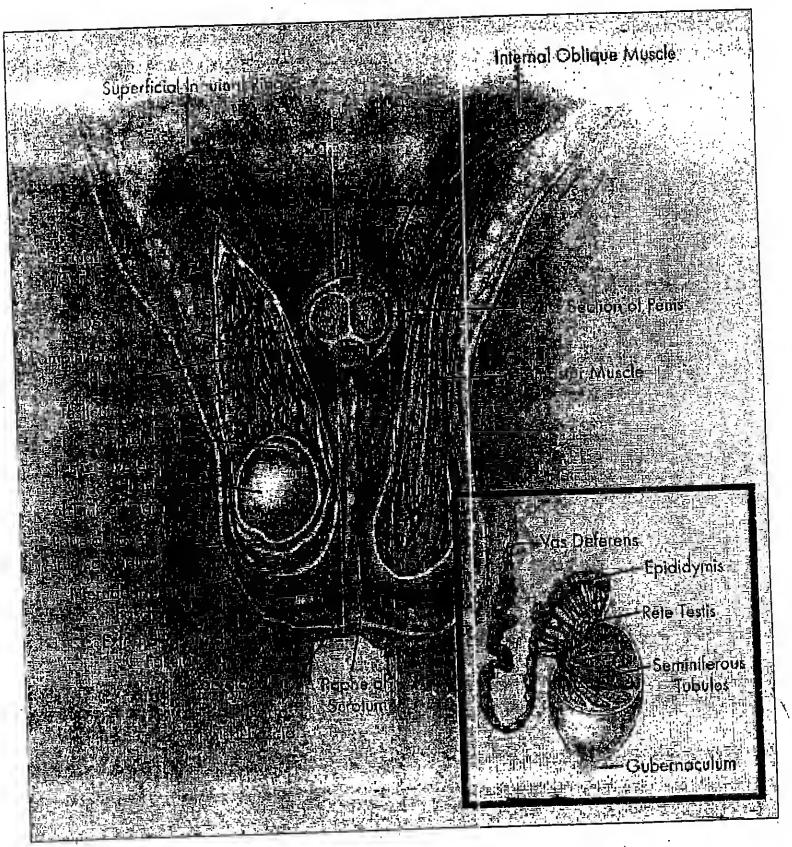


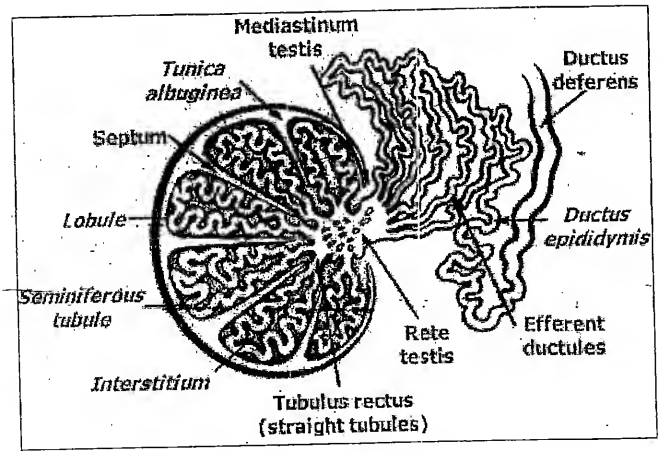
Fig. (22): Process of fertilization (1a) deposition of normal spermatozoa; (b) cervical mucus penetration; (2) sperm migration through the uterus; (3) sperm capacitation and hyperactivation in the fallopian tube; (4) an enlargement of spermatozoa-oocyte interaction (5) sperm penetration of cumulus oophorus; (6) sperm binding to zona pellucida; (7) acrosome reaction; (8) sperm penetration of zona pellucida; (9) sperm fusion with oocyte plasma membrane; (10) cortical granule exocytosis; (11) nuclear decondensation; (12) nuclear fusion (*Tripp and Gagnon, 1997*).

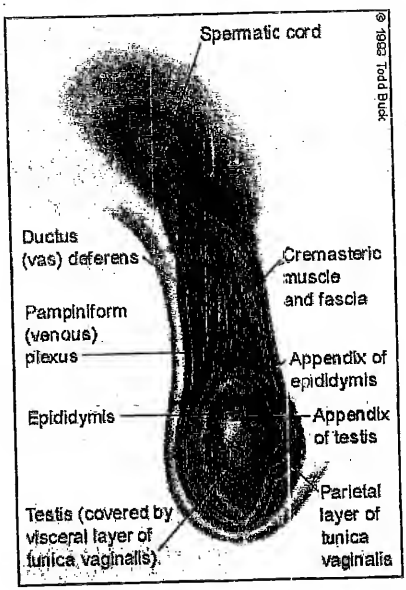
Prader orchidometer

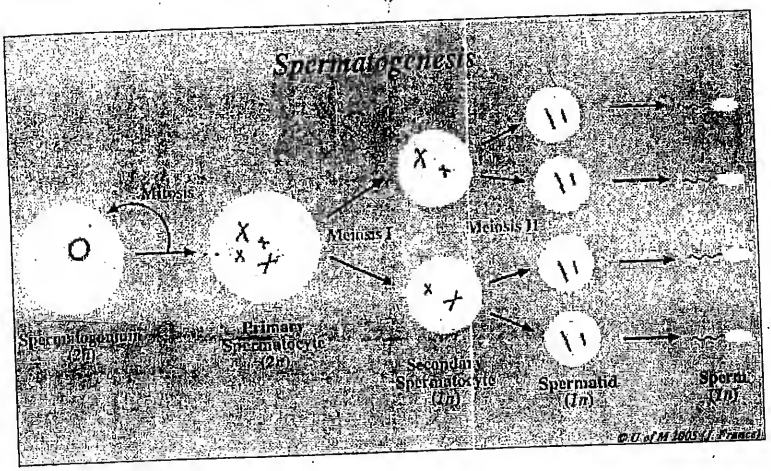


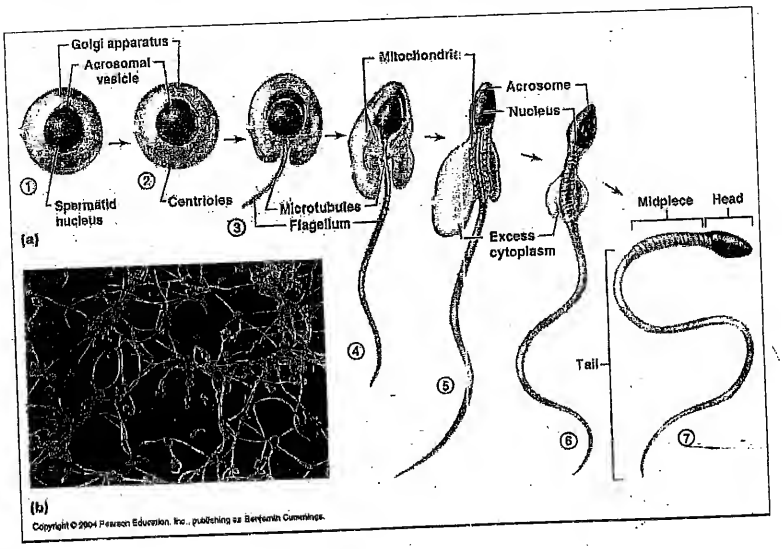



Internal Oblique Muscle
Section of Penis
Muscle
Vas Deferens
Epididymis
Rete Testis
Seminiferous Tubules
Gubernaculum
Septa of Scrotum


Mediastinum testis
Tunica albuginea
Septum
Lobule
Seminiferous tubule
Interstitium
Tubulus rectus (straight tubules)
Rete testis
Efferent ductules
Ductus epididymis
Ductus deferens


Spermatic cord
© 1982 Todd Buck
Ductus (vas) deferens
Pampiniform (venous) plexus
Epididymis
Testis (covered by visceral layer of tunica vaginalis)
Cremasteric muscle and fascia
Appendix of epididymis
Appendix of testis
Parietal layer of tunica vaginalis


Spermatogenesis
Mitosis
Meiosis I
Meiosis II
Spermatogonium (2n)
Primary Spermatocyte (2n)
Secondary Spermatocyte (1n)
Spermatid (1n)
Sperm (1n)


Golgi apparatus
Acrosomal vesicle
Mitochondria
Acrosome
Nucleus
Spermatid nucleus
Centrioles
Microtubules
Flagellum
Excess cytoplasm
Midpiece
Head
Tail
(a)
(b)
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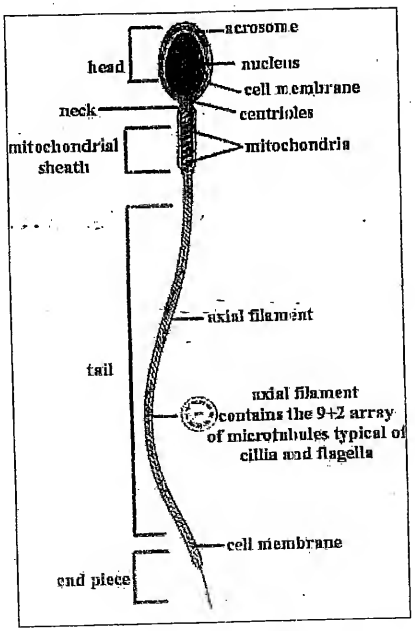
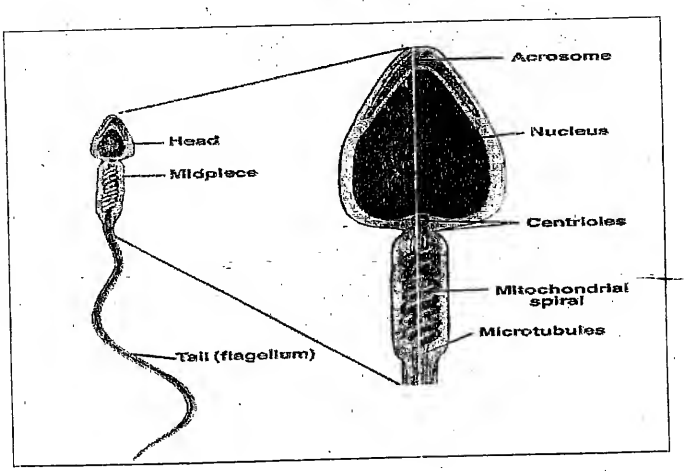
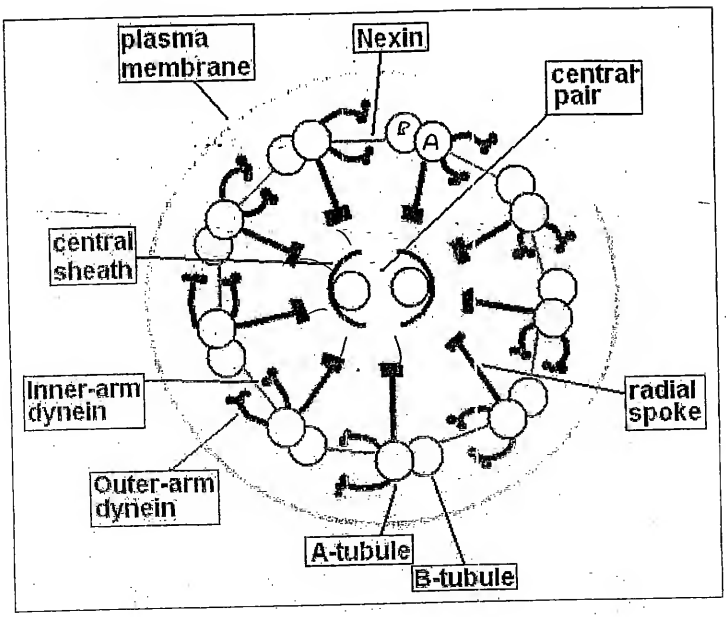
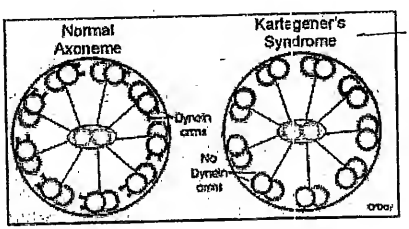

acrosome
head
nucleus
cell membrane
neck
centrioles
mitochondrial sheath
mitochondria
axial filament
tail
axial filament contains the 9+2 array of microtubules typical of cilia and flagella
cell membrane
end piece


Acrosome
Nucleus
Head
Midpiece
Centrioles
Mitochondrial spiral
Microtubules
Tail (flagellum)

Axoneme structure



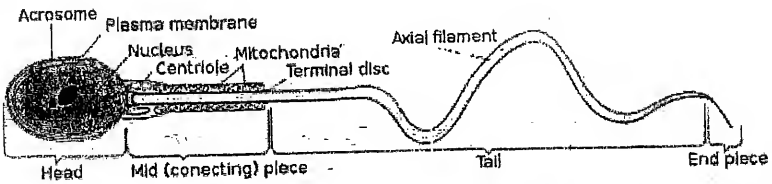


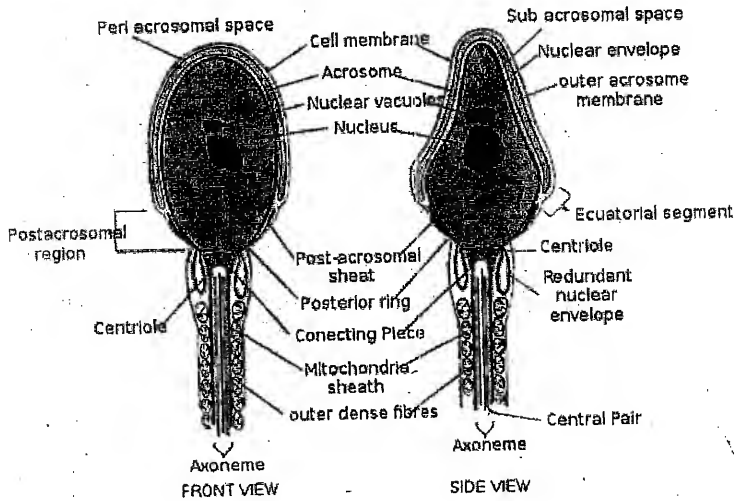
No Nexins

No dynine arm.

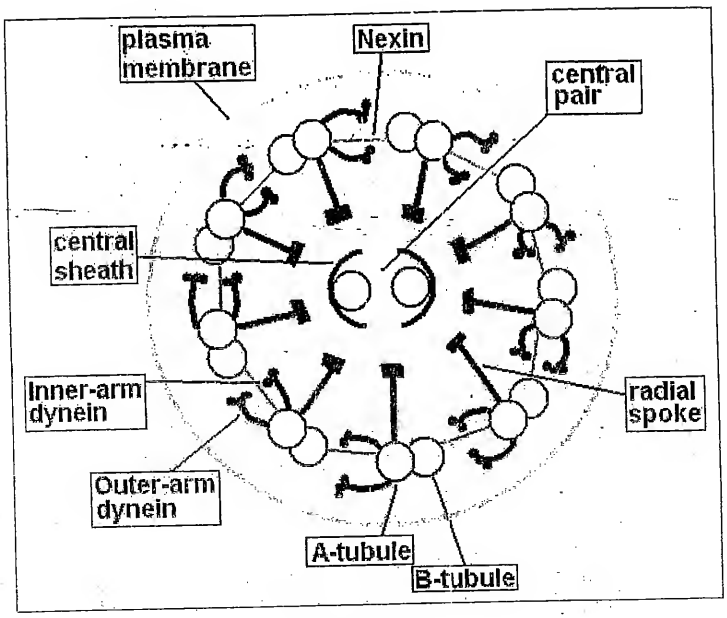
9+0 synd = No 2Central Single Microtub.

Sperm structure



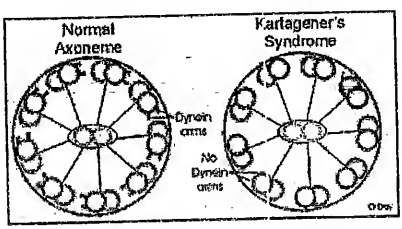


Axoneme structure



2 Abnormalities

1. Kartagenes Synd = Absent dynein arm & Nexins



2. 9+0 Synd : Absent Central Single Microtubule

physiology of the Testis

1. structure
↓
Testis is suspended by The spermatic Cord in side The Scrotum.
Testis: SP. Cord > Scrotum

2. functⁿ
↓
Later on.

3. Regulatⁿ & Control of functⁿ
↓
Later on.

TRE

(structure:)

Testis
① structure & anatomy.
② Coverings.
③ Vs.
④ Histology.

Spermatic Cord
↓
Contains:
3 facial Layers
3 vascular structures
3 non " "

Scrotum
↓
Contains 3 Layers
. Skin
. facia.
. dartos m.

① Testicular anatomy & structure

it has
③ dimension. (SBB) &
③ Coverings: (3 Tunica)
③ Intratesticular ducts: (TRE).

oval
. dimensions: length 4.5 (3.5 - 5.5) X 3 X 3

Size
. Volume 15 - 25 ml (size) < orchidometer L, W (special Caliber) W² X L

. axis & relation: → (ch. 24)

. Inner structure: The inner aspect of Tuni

vertically in Scrotum

NB Testicular L < 3 → Hypogonadism

Clinical Anatomy

L = 35-55
W = 25-35
Height = 15-25

Volume = LXWXHX 0.5-2

Endocrinology

Orchidometer

⇒ Testicular Volume (Normal)

- Prepubertal 1-3 ml
- Pubertal 4 ml
- Adult 12-25

Test. Size : 1st Sign of pubarty

Macroorchidism
- MR
- fragile X Synd.

① Interstitial : 15% of Test Vol → 18-20% in occupied by Leding

② Tubular Compartment : 60-80% of Test. Vol.
- Contains < Germ Cells.
  2 Sertoli cells

Albuginea gives many septae. That divided the testis from the ant. aspect to the post. mo. These septae then converge at posterior asp. of testis to form a mass of fibrous tissue known as mediastinum testis which acts as a supporting structures to the testicular vs & ducts that pass through it.

So that the parenchyma of the testis is divided by these septae into 300 tubules each lobule contain (1-3) Seminiferous tubules (Tubular Component). Are Convoluted

These Seminiferous tubules: (3)

1. Surr. by (3) Layers: Collagen, Myoid (Myoid Cells), Adventitial C. (fibroblasts)
2. Contain → 2 Cells: Germ, Sertoli — Rest on B.M.
3. Each Convoluted Semi. tubule → "ends" 2 Straight Tubuli Recta (T) → enter Mediastinum to form Rete testis (R) → Then form the efferent ductules (10-12) → Then enter the head of epididymis forming epididymal lobules that coalesce to form a single epididymal duct

each Seminiferous tubules Composed of: (Up)

- B.M on w. rests: <
- Germ cells
- Sertoli Cell.

Germ cells: These cells rests on the B.M & give rise to the Spermatogenic Cells that include different types of Cells arranged in an organized pattern of maturation from the most primitive type of cells on B.M to most advanced types of Cells near the lumen of Seminiferous tubules.

These Cells include: (form below to up, sequential Maturation).

1. Spermatogonia → (2) types
2. primary Spermatocytes → (4) phases
3. 2ry Spermatocytes.
4. Spermatids → (6) types
5. Mature sperms.

No of Germ Cells = 13

Spermatogonia include (2) type: A, B

- AL → type A Long → (1) in frequent.
- AD → type A dark → inactive
- AP → type A pale → inactive (give Title to)

Type B spermatogonia.

↓ (Mitosis)

Primary Spermatocytes

4. Primary Spermatocytes are (4) phases:

- P-L : Pre/Leptotene
- L : Leptotene
- Z : Zygotene
- P : Pachytene → 2ry Spermatocytes

Short Lived & difficult to be identified in Histological sections.

give rise to

- Spermatids:- (6) Types:
  - Sa, Sb1, Sb2, Sc, Sd1, Sd2.

Then Spermatids are undergo Morphological changes into the mature sperms.

Sertoli Cells = Sustentacular (or) Supporting cells. (Nurse cell of testis) = Mother cells.

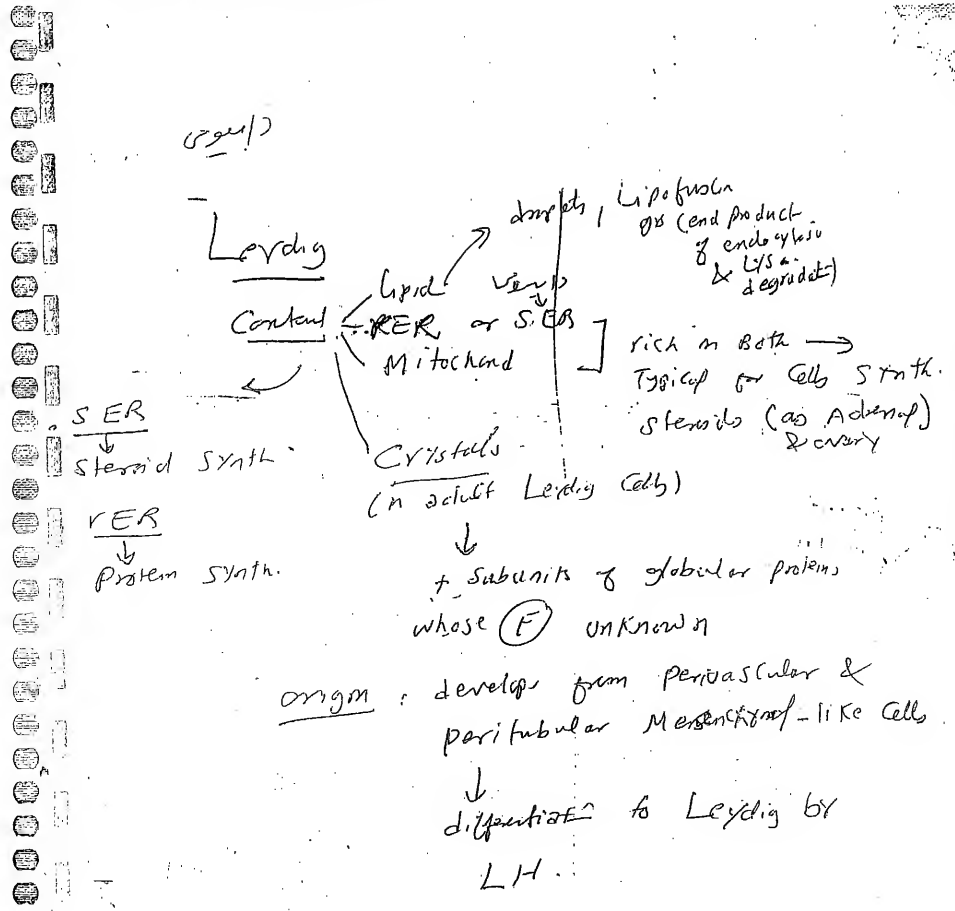
- Tall cells, irregular nuclei, rest on the BM of the tubules & send their prolonged cytoplasmic projections toward the lumen of the tubules.

Sertoli cells make 2 relations:

1. Sertoli Cell - Sertoli Cell relationship.
2. " " - Germ Cell "

- Sertoli Cell - Sertoli Cell relationship: Tight junctional Complexes exist bet. the adjacent sertoli cells → formation of blood testicular barrier .. ω is not formed before puberty
- This barrier divides the Seminiferous tubules into 2 compartments (1) basal Compartment ω contains the early stages of Germ Cells (spermatogonia, young spermatocytes)
(2) Adluminal Compartment ω contains the late stages of Spermatogenesis (mature spermatocytes & spermatids)
- The barrier has both metabolic (F) (Metabolic protective) functions (by Controlling the passage of materials through it) & Protective Immunological functions (by prevention of passage of the antigenic sperms into the blood & Lymph Vs ω → Anti-sperm antibodies response. (Immunology)

Sertoli Cell - germ cell relationship:
There is a physical Contact bet. the 2 cells, ω may help in the organized pattern transport of the germ cells in an organized pattern toward the lumen of the tubule by the specialized changes in the configuration of Sertoli cells
Finally, the late spermatids are found inside recesses (invaginations) of the apical portions of the Sertoli cells. They obtain their


Leydig
Content
Lipid droplets
Lipofuscin (end product of endocytosis & Lys degradation)
SER
RER or S.ER
Mitochond
rich in Both → Typical for Cells Synth. steroids (as Adrenal & ovary)
SER
steroid synth.
Crystals (in adult Leydig cells)
RER
protein synth.
+ Subunits of globular proteins whose (F) unknown
origin : develop from perivascular & peritubular Mesenchymal-like cells
differentiate to Leydig by LH.

⑥ Maturation by losing their cytoplasm that's phagocytosed by Sertoli cells; finally they are released by the process of (spermiation) from the re cesses of Sertoli cells.

Interstitial Compartment of the testis:

The space bet. the tubules .. Contain:

1. Blood & Lymph Vs / INs.
2. fibroblastic supporting cells.
3. Macrophages
4. Leydig Cells.

Bvs & lymph, fibroblast & Mac, Leydig

Shape: Polygonal. Rough endoplasmic reticulum. Eosinophilic inclusion

Nuc: round & vesic. Cytop: Eos.

Contain: SER, lipid droplets, Reinke crystals. (lipo-)

- Present in clusters
- Account for 5-12% of the testicular Vol.
- Each cell has around nucleous & prominent Mitochondria, SER, Reinke Crystals & lipid droplets.

(Fun) => Source of testosterone

Coverings of the testis:

"2 Layers"

① Tunica vaginalis: formed of outer parietal Layer & inner Visceral Layer. the space bet. The 2 Layers contains some clear fluid that may be pathologically (↑) in cases of Hydrocele.

② Tunica Albuginea: Tough fibrous Layer (formed) Collagen + Smooth m. cells that give the testicular Capsule a contractile activity & helps to regulate flow of Testicular fluids.

Contract act in cold

Functions of Sertoli Cells = Mother or Nurse cells = Sustentacular Cells Exo

(Support of Spermatogenesis).

Support & Regulate Sp-

Regulate ③ ↓ Physical regulat" ✓
Chemical "
Immunological regulat"

Supporting Cells

① physical reg. of spermatog. ≤ Anchoring / Transport / release of Germ cells.

The physical Contact bet Sertoli Cells & the developing germ Cells (≤ anch. transport release), help the propagation of germ cells from the base toward the lumen of Seminiferous Tubules, finally, The mature sperms are released from (recesses = invaginations) in the Cytoplasm of Sertoli Cells into the seminiferous tubular lumen by the process of "Spermiation".

(Anchoring → Transport → release by spermiation).

All these processes of physical Contact bet Sertoli & germ cells are dependant on 3 groups of specific structures of Sertoli Cells as following:

① Cell Skeleton structures: e.g. actin filaments & microtubules that help morphological changes of Sertoli Cells & their anchoring of germ cells.

② Cell Surface Structure: Cell Junctions: → Blood testis barrier
invading
Examples are; Cellular Junctions (that form blood testis barrier) & invading processes. (that anchor the germ cells to Sertoli cells)

③ Cell adhesion molecules: examples include Cadherins & Integrins that help Transport of germ cells along the Sertoli Cells.

① Cadherins: → Transp.
② Integrins:

SF

Free iron

- Needed for cell division
- Transferrin in Semen

Correlate ↑ Seminal parameters including ↑ Sperm density

- SPARC
  - involved in Ca^{+} Transport
  - regulate [TGF-β → differentiate ↑ Sertoli
    → Matrix-Metallo-proteases (involved in cell adhesion.)
    → disrupt Collagen → disrupt BTB
- CRAB
  - Retinol (Vit A):
    - maintains BTB
    - ++T effect on Sertoli
    - helps adhesion ↑ Spermatogenesis
    - N.S

Sertoli cell Reg. ↑ Testis development

② Chemical regulat of spermatogenesis

The secretory products of Sertoli cells that can regulate spermatogenesis can be divided into ③:

- Transport & binding protein
- Proteases & anti proteases
- Growth factors

Transport & binding protein

examples:

① ABP (androg. binding ptn): it transports androgens to germ cells (androg required for spermatog.)

② Glutamyl Trans-peptidase for a.a.

③ Retinol binding ptn for V.t.A (RBP)

④ Transferrin for Iron

⑤ Ceruplasmin for Copper

⑥ SPARC (secreted protein that is acidic & rich in cystine ... for Ca^{+} transport into germ cells ... ω is needed for spermatogenesis.

ABP, RBP, SPARC + ζ (Fe, Cu)

ABP RBP Fe Cu Ca Ca

Proteases & anti proteases

② Proteases induces controlled proteolysis (ω) is needed for Sertoli cell remodelling & germ cells movement & spermiation

① Anti proteases: help to protect the cells from some effects of proteases.

proteases / Anti proteases

Growth factors

MIS (Mullerian Inhibitory Subst) — Activin — Inhibin — IGF — IL1 — IL6

These factors control cell/or structure & functions.

How by 2 mechanisms.

Examples of these factors.

① MIS (Mullerian Inhibitory Subst) ↓ has 2 effects: prenatal on Mullerian ducts; postnatal: on spermatogenesis

② Inhibin & activin:

- inhibin: secreted from sertoli in response to FSH ↗ → ⇒ ⇐ FSH ↙ spermatogenesis.
- Activin: it has antagonistic action to inhibin

③ IGF-1 (insulin-like growth factor 1) & IGF: Transforming Growth factor

④ Cytokines (IL-1 & IL-2)

How these growth factors: Control Cellular functions

Structures: → By ② Mechanisms

Systemic Control

① Neurologic Control: through Nervous System.

② Endocrine Controls Through Hormones that reach via Blood.

Local Control.

① Autocrine Control (Cells) Produce Chemical Substances that act on the same Secreting Cells.

② Paracrine Control: The Chemical Substances that are secreted act on adjacent Cells.

③ Immunological Protection: → Blood testicular barrier of spermatogenesis.

This function of Sertoli cells depends on the blood testicular barrier & TGF-β (Transforming growth factor-(β)) that has immune protective functions for sperms both inside the testis & inside the vagina as it is activated in acidic pH of vagina.

Physiology.

By anchoring, Transport, Release

By

Chemical

C1: ABP
C2: R3F
C3: Transf. Fe
C4: Ceruloplasmin
C5: SPARC

Proteases, anti n

Growth factors

Immunological

NIS

Activin

Inhibin

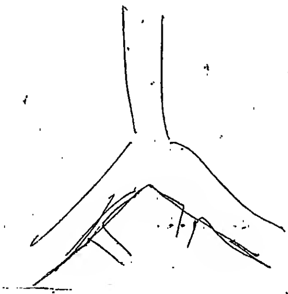
IL1

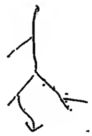
IL2

IGF-1

TGF1

Thymo





Blood supply (TVC)

1. Aorta
below Renal a.
(Testicular)
Mediast.
(Net work)
Test. supply upper epid.

2. int. iliac
Vasal a
from sup. vesical
tail by network that supply test.
VW lower epi.

3. Ext. iliac
Cremastic
from inf. epig.
T. Ab.
BY anast. c other aa.

TVC

TVC

③ Tunica Vasculosa (The inner Layer of T. Albuginea)

- is highly vascular
- Accordingly, Meticulous surgical hemostasis should be done during closure of T. Albug. during open testicular biopsy to avoid intra-testicular Hge

Testicular Vs { Arteries / Veins / Lymphatics }

Testicular arteries: → ③

Testicular art. (internal spermatic a.)
- the main a. of the testis
- br. from the Aorta just below the renal a.
- Crosses the ureter supplying it, Then enter the inguinal canal in the spermatic cord
- Supplies the testis & convoluted network of vs through the mediastinum testis.
- gives br. to upper part of epididymis.

Vasal (deferential a.)
- br. from iliac inf. vesical a.
- Passes inclose contact to Vas supplying it.
- tail of epididymis by supplying the Testis by Capillary Network

Cremasteric a. (ext. spermatic a.)
- br. from inf. epigastric a.
- Passes along the sheath of Sp. Cord
- T. Vaginalis by anastomosing other arteries.

Testis → upper pole.

Vas → end at the upper pole supplies it

(3)

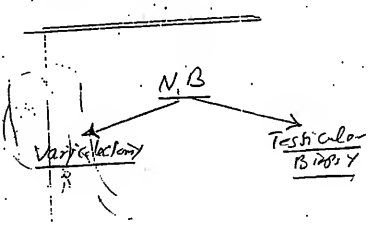
Surgical important Points:

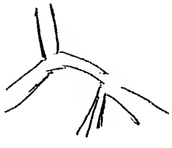
① Varicocelectomy & Testicular artery:

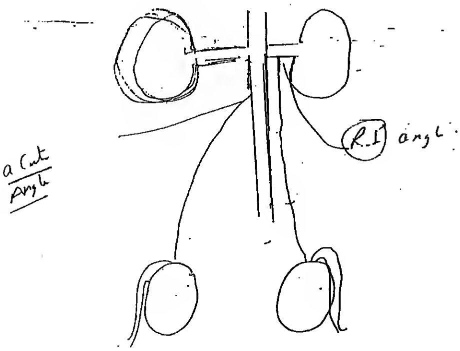
The rich arterial supply to the testis through 3 different arteries è rich anastomosis bet. them helps to prevent testicular ~~atrophy~~ atrophy if The testicular a. was accidentally injured during varicocelectomy. However this is more physiological to preserve it during the operation.

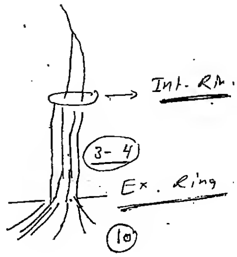
② Testicular Biopsy & Testicular a.

The recent performance of multiple sites biopsy may be associated è accidental inj. of the testicular a. branches inside the testis (see testic. Biopsy)... This may be prevented by performing the least possible incisions during the procedure that should be also in the last vascular area ie are the medial & lateral upper poles of the testis.









Pampiniform plexus

emerge from back of testis, Receive Tributaries from epid → form Convoluted plexus (main mass of cord)

ascend in cord, infront of the Vas

at ext. Ring → unite → 3-4 veins at int. Ring 2 veins → ascend → single vein.

Testicular veins → 3 groups.

① **Ant. groups** (Testicular veins)
They are 10 veins that anastomose to form the pampiniform plexus of veins which is closely associated with the testicular a.
The brs. are ↓↓ in No gradually till they pass through the ext. inguinal ring where they become the single testicular vein.
The left testic. vein (spermatic) drains into → left renal vein Rt angle
the R. " " " " " → IVC. acute angle

② **Middle groups** (Vasal or deferential vein)
The vasal veins & The funicular veins that accompany & drain the vas & epididymis
Vas deferens → drain into (prostatic plexus) → (internal iliac)
& (vesical plexus.) / // orchidometer

③ **Posterior group** (Cremasteric veins):
The cremasteric veins become separated from the spermatic cord at the ext. inguinal ring & drain into → (Inferior epigasteric) vein → IVC / Ex. iliac

Surgically important Points:
There is anastmotic sites bet. the right & left venous systems at the level of inguinal region,
(testic) ant → ... vesical & prostatic plexus → int. iliac
(vasal) mid →
post → inf. epig. → ext. iliac

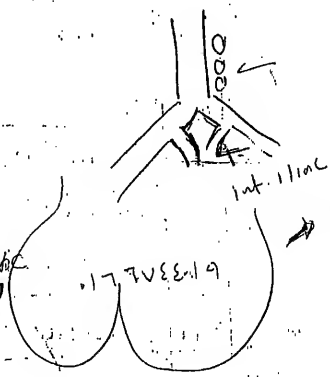
So that: Varicocelectomy should be done at inguinal or supra inguinal level to prevent The abnormal Venous reflux in both R & Left Sides.

Testicular Lymphatics:

Intra testicular Lymph ducts originate in The testicular interstitium & ascend inside the spermatic cord to drain finally into Para aortic L.Ns at Lumber region (In the testis develop during the intra uterine Period).

So that testicular malignancy will give rise to paraortic metastasis inside the abd. & not to inguinal region.

① Testis → Paraortic
② Coverings → inf. iliac L.Ns
③ Scrotum → inguinal.



Nerve supply (Vas. Sympathetic supply)

Sperph & Para Symp

(T10, 11)

The spermatic Cord:

③ → Facial Layers. ③
Vascular Structures. ③
non " " ③

Ⓐ Facial Layers ③: The Cord includes 3 facial layers that are derived from the ③ Layers of the ant. abd. wall:

① ext. spermatic facia: → derived from ext. oblique aponeurosis.

② Cremasteric ms & facia: derived from Int. oblique & Transversus abdominis mscls.

③ Internal spermatic facia: derived from Transversalis facia.

Ⓑ Vascular Structures ③: 3 aas, 3 veins & Lymphatic

Ⓒ Non vascular structures ③: vas. Cremasteric m. Testicular nerves:

Super spermatic Nerve derived from < Renal / Inter mesenteric plexus

So. UT disease → Test. pain

arises from thoracic (10 & 11) & may be from the first Lumbar & pass ē the testicular artery Through the inguinal Canal then inside / sp. Cord supplying the tunica albuginea ē autonomic pain receptors while testicular parenchyma itself devoid of pain receptors.

* Clinically important points: d.t high origin of Testicular nerves, the Testicular pain may be referred to / upper Abd.

No Somatic / Nerves mainly restricted to small inters...

Function of The testis.

2 → Spermatogenesis (Germ cells / Sertoli cells)
Steroidogenesis → Leydig cells.

* Spermatogenesis occur by Germ cells while support of spermatogenesis occurs by Sertoli cells.

* spermatogenesis → 3 phases:
  - prolif. phase
  - Meiotic
  - spermiogenesis

* **prolif. phase**:
stem cells (double no of chromosom) (spermatogonia) → prolif (mitotic) → Type (A) spermatogonia (inactive A / A / A → Active) → Type B spermatogonia → 1ry spermatocytes (2N)

* **Meiotic phase** → :
1ry spermatocytes double no of chr (4N) → replicate DNA so contain chromosome diploid no (46/4N)
1st Meiotic division → 2ry spermatocytes (diploid no of chr.) → 2nd Meiotic → Spermatids (haploid no) 23 chr
Zygote (46) ← 23

* **Spermiogenesis phase** → Maturation changes occur in spermatids → Mature sperm.

These changes Include:
3 Format of Acrosome.
tail.
Nuclear & Cytoplasmic changes.

→ Spermiogenesis

- **Formation of Acrosome** the acrosome is a modified bag contains Lysosomal Enzymes that are necessary for penetration of ovum, Golgi apparatus, gives the material for acrosome formation.
- The formation of this acrosome passes by (6) stages (Sa, Sb1, Sb2, Sc, Sd1, Sd2) to form the acrosomal cap at the anterior pole of the sperm. Pushing the nuclear peripherally.

* **formation of the tail** → "تكوين الذيل"

→ The centrioles migrate to the other pole (peripherally). To form the tail, at the same time the mitochondria migrate to the same pole & arranged spirally around the tail to supply the sperm (E) energy. (source of energy).

* **Nuclear & Cytoplasmic changes** → تغيرات

- as a result of formation of the acrosome the acrosom. cap pushes the nucl. peripherally so become flattened (E) Condensed chromatine.
- The Cytoplasm is ↓↓ (to) form the "residual body" That is phagocytosed by the Sertoli Cells. to complete the process of formation.

Lons ① Nucl.: pushed peripherally condensed chromatin
② Cytopl.: ↓↓ → Residual body

clinically important points

Time needed for complete spermatogenesis from (AP) tail formation of mature sperms is about 74 ds

+ 16 ds needed for maturation in epididymis تقريبا
= 90 ds (so time needed for any th in 1st course = 90d

NB

- No of sperms produced daily

  70 million

Sertoli

- Nucleus → Chic Tri partite
- BM < laminin, Type IV Cell — formed by < Sertoli Cells & Myoid Cells
- Cytoplasm . extensive Golgi app.
  . lots of RER . SER & lipid droplets (C correlating to steroid synth).
- Cytoskeleton :
  - Actin filament (some spermatogonia)
  - Intermediate (Mainly Vimentin) (protect against Mechanical forces)
  - Microtubules → directionalized Transport in Cells.
  - assist to Maintain Architecture
  - entrenchment & then release of Spermatids

Tubulobulbar Complexes & Ectoplasmic Specialization

Control (Regulation) of Testicular Function

(Nieschlag 2010)

Endocrine = Central or Hormonal Control

(BY) Hypothalamic-pituitary axis or Hormones → play central role in regulation of testicular Function.

- GnRH
- FSH
- LH
- Prolactin

Paracrine / Autocrine = local regulation or Control

important for (modulation) of Endocrine (Hormonal) Activities by certain locally produced Factors

- Steriod Hormones
- INSL-3
- Growth Factors
- Immune System Factors.

Central Endocrinal Control (HL) Nieschlag

- GnRH
  - Structure
  - Secretion
  - Mechanism of Action
- Gonadotropins:
  - Structure
  - Secretion
  - Mechanism of Action.
  - Role of FSH & LH in Spermatogenesis.

VIAGRA
sildenafil

Control of Function of Testis (2) spermatogenesis / steroidogenesis
is controlled by 2 mechanisms: pretesticular control / Testicular control

* Pretesticular control: by Hypothalamic Pituitary Testicular interaction.

Hypothalamus: sec. → GnRH = LHRH (gonadotrophin releasing H) from arcuate nucleus, secreted at pulsatile manner every (60–90 min)

↓

Pituit: ++ Release of LH & FSH from Pituitary (pituitary gonadotrophins)

Pituit: FSH, LH, Prolactin

(FSH) ↓ action: Sertoli cells ↓ ++ Sec. of
- Androgen binding protein ↓ Bind to Andr. secreted from Leydig cells → High conc. of And.
- Inhibin ↓ inhibit FSH release from Pituit.

(LH) act on Leydig cells ↓ Androgen ↓ Germ cell maturat.

N.B.: FSH & LH acting by activat. of "adenyl cyclase syst."

Role of PRH ?

Prolactin: PR
- Physiological NL level → ↑ conc. of cholesterol esters → potentiate eff. of LH on Leydig
- Pathological high level → Impaired Testicular functn either By:
  - ↓ pituit response to GnRH
  - or ↓ 5-α reductase activity.

① ~~Regulat~~ Control of Test funct — by axis → Regulate

②

Testis

Structure & [illegible]

funct

1. what its funct → spermatogenesis → f of Sertoli
2. Control of its f → axis
3. factors affecting its funct

Testis → Androgen .. (Gonadal Hs) ..

Regulat. of this axis or Control

+ve (stimulatory) feed-back

• Hypoth. Hs → + Pituit Hs → ++ Gonadal Hs

(↓LH) GnRH.

Gn ↑ (LH) ↓Leyd ↓FSH.

− ve (inhibitory) feed back

(a) Aromatizat. of Gonadal Androgen to Oestrogen: → (−) GnRH release → (−) LH

(b) Inhibin → − − FSH release.

Factors that aff. the testicular function

1. Age
2. Temp
3. Vasculat. ure

Aging aff. Sexual response cycle, Testicular function ..

↓ effect is: « on »

Germ cell S.NT

(at 40 y.o → 85%) of ! Semi-nef. tubules show NL spermatogenesis. —

(after 70 y.o → 30%

after 80 y.o → 15%

∴ ! tubules show
↓ Volume ..
↑ thickness of wall
↓ sperm product

Sertoli

at young adult ! testis contain 500 million Sertoli cells

↓

after 50 y.o. ↓ by 50%

Leydig

after 50 y.o.

1. ↓ No by 50%
   ↓ Testosterone
2. ↑ SHBG
   ↓ free testosterone
3. ↑ peripheral conversion of And. to Oest.
   ↓ A/O Ratio

↓ No (SHBG) Testo (A/O) ↓A/O

BPH. Gynaecomastia in old.

Temp

Testicular Temp is (34) °C

- 3-4 °C below Body Temp
- 1.5-2.5 °C below scrotal Temp.

34 °C

Reg. of Temp by .. (2 factors)

2 mech.

4

Thin .. No S.C. f...
Rich in ~~blood supply of~~ Sweat gland
Large surface area that
Controlled by dartos (relax. & cont.)

(A) Scrotal skin that

(B) Counter current heat exchange depend on specialized arrangement of pampiniform plexus of veins ... that cools the blood before reaching the testis ... (by surrounding coiled veins.) *

low temp ... is essential ?? because

A B 2 Cells (1) Spermatocytes & Spermatids are Heat Sensitive & degenerate at low temp.

2 ptn (2) Some ptns as Androgen binding ptn & ptn G (that is essential for FSH function ...) Are active only at 34 °C.

A C Epididym

2 (•) 2 pathological conditions are Ass. c disturbance of Testicular funct. d.t. disturbance in Temp are
→ (•) undescended testis
→ (•) Varicocele ....

3 Vasculature (microcirculation) → despite the Total testicular blood flow is constant .. There is a great variation in the flow among the different regions of testis ... & According to the dilatation & constriction of Terminal arterioles ...

* Exact mechanism by w. testicular micro circ. may aff. the testicular functions is Not yet established

* many studies state that the circ. may play a role in infertility & possible Role of chronic Testicular ischemia in

(NEW!) . Local Regulation of Testicular Function

(Paracrine / autocrine regulation)

- There are (3) Types of local interactions & communications bet. different testicular cells which are mediated by "Certain Secretory Factors":
  1. Paracrine: Factors acting between Neighboring cells (by diffusion)
  2. Autocrine: factors which are released from the cell & work back on the same cell.
  3. Intracrine: Factors never leave the cell & its site of production & action is the same.
- Sertoli cells were viewed as coordinators & regulators of Germ cell development & maturation; (Recently) they are now believed to be influenced by Germ cell products that can influence the secretory activity of Sertoli cells.
  Eg: Sertoli cells are under the local control of germ cells
- These Paracrine / Autocrine / Intracrine factors mediate the communication between:
  - ✓ Interstitial & tubular compartments
  - ✓ Sertoli & Germ cells.
  - ✓ Germ cells & " ".
- these factors include:
  1. Steroid Hormones
  2. INSL-3
  3. Growth Factors
  4. Immune factors.

1. **Steriod Hormones:** T is the main secretory product of Testis.

T.

(Intratesticular Conc. is 80 fold > Serum T.)

it acts through 3 ways

- as T.
  - ↓
  - affect Spermatogenesis (mainly Needed for initiation or Spermatogoniogenesis).
- as DHT (under effect of Testicular 5-α reductase)
  - ↓
  - No detected role in spermatogenesis. (So Dutasteride no effect on semen)
- as Estradiol (under effect of Testicular Aromatase).
  - ↓
  - it's deficiency has no role in spermatogenesis.

T. Receptors found in { Sertoli Cells, peritubular myoid cells

Leydig cells (T) ↓ Sertoli Cells ↑↑

1. Sertoli Cells: Secrete ABP ↑ binds T. in the interstitium → prevent its rapid metabolism to E. & transport it inside SNT to maintain High Conc. Needed for Spermatogenesis.

FSH ↓ ABP ↓ T Tubules ↑ Cadherins ↑ FSH ↑↑ ↑↑

2. FSH reinforce T. Action (& FSH recombinant → ↑↑ T. product) ↑.
   transplant of germ cells

3. T. → ++ Cadherins product ↑ by Sertoli Cells
   (So if ↓ T → affect Spermatogenesis)

FSH++ ⟷ ++T

2. **Insulin like Factor-3** ---

- relaxin-like proteohormone produced by Leydig Cells.
- May play a role in regulating Spermatogenesis.

3. **Growth Factors:** e.g. by Sertoli cells:

transforming growth factor

- IGF-α & β (α → ++ Testis / β → --)
- Inhibin → ++ Spermatogenesis (Recently Serum level Correlate ∝ Spermatogenesis & Testicular size) → So used as Endocrine indicator of local spermatogenic defect.
- Activin → ++ " "
- IGF-I, EGF → ++ Spermatogenesis.

4. **Immune System Factors:**

- Some Cytokines produced by: WBCs, Macrophages & Mast cells → affect Spermatogenesis e.g.
  - TNF
  - Leukemia inhibiting factor (LIF) → role in Sertoli-Germ interact°
  - IL-1 < 2
  - SCF (stem cell factor, produced by Sertoli)
  - MIF (Migration Inhibition Factor produced specifically by Leydig cells).

Spermatogonial differentiation & development.

TNF, IL-1, LIF, MIF, SCF

Epididymis

(Epi= above / didymos = Testis)

Anatomy (4cm L / tubules = 4m)

Coma shapped structure that overlies the Sup- & post-lat. Aspects of testis.

— formed of 3 segments:

(1) Head (Caput) : at upper Pole of testis
(2) Body (Corpus) : at post aspect of testis.
(3) tail (Cauda) : attached to inferior pole

- Length < 4cm uncoiled tubule : 6 meter

- Structure

(1) Head: formed of 12-20 tubules (Efferent ductules) that arise from Rete testis those tubules are very convoluted to form conical masses called lobules of epididymis. 20 together form the head.

Each lobule Composed of single Convoluted duct ≃ 20 cm Long.

duct of epid. encapsulated by fibrous sheath that sends septa → divide the duct into histologically similar areas.

(2) Body & tail: Efferent ductules → become one duct called (Epididymal duct)

This duct very convoluted at body & tail end called Epid. tail → Vas deferens.

- Blood supply : testicular, Vasal & Cremasteric artery
- Venous drainage : to pampiniform plexus of veins.
- Nerve supply : Sympathetic
- Lymphatic drainage: Head & Body (as testis) → Para-aortic L.N.
  tail (as VD) → External iliac L.N.

Function of Epididymis:

4 Main functions

1. Sperm Storage & Concentration
2. Sperm Transport
3. Sperm protection
4. Sperm Maturation

1. Sperm Storage & Concentration
   - ejaculated sperms enter epid. & pass through
   - Sperm concentration ability of epid. is due to fluid reabsorption subsequent to antiluminal Electrolyte transport...

2. Sperm Transport
   - Epididymis act as a duct that transport sperms via:
     (1) Hydrostatic pressure from fluid secretion in testis. (by Sertoli)
     (2) Motile Cilia of epididymis.
     (3) Rythmic Contraction of Myoepithelial cells.

3. Sperm protection

4. Sperm Maturation

(A) sperm < Conc. / Storage / Transport
(B) Protect.
(C) Maturate.

[3] Sperm protect: through secret of:

Anti-protease → (A) Protease inhibitors: (-- proteolytic degradat-)

Antioxidant → (B) Anti-oxidants (Glutathione peroxidase)

Be barrier → (C) Blood Epididymal barrier (Helped by Cadherins)

Anti- → (D) Suppressive factors (↓ Lymphocyte & Complement activat-).
↓
immunosuppressive

[4] Sperm Maturat- (W)

Means 2:

- **Attainment of Motility** [Sperms of tail have Max Velocity > that of Head of epid.]
  - Some epidid. products are secreted have role in Sperm Motility:
    - (1) L. Carnitine: protein that, not synthesized by epid. but Concentrated From circulat- inside epididymis.
    - (2) α-glucosidase: enz
      - secreted from Epid.
      - a Marker for ~~[illegible]~~ epididymal
      - (age) of Epididymal Obst.
- **Attainment of Fertilizing Capicity** (Fertilizing ablity of Sperms & tail > Sperms of Head & bod) through sec. if:
  - (1) Zinc
  - (2) Sialic acid: essential For integrity of ~~[illegible]~~
  - ess. For Maturat- (3) Inisitol
  - (4) Glycerol Phosphocholine
  - (5) D. Galactosidase (++ Spermatorum binding)
  - (6) appearance of Maturational Antigens:

. Maturational Ags. are surface structure found only on Epid. as

① P34H protein (sperm ovum interact protein - IVF succn depends on sperm content of it).

MMP ② Major Maturational protein (MDS2)

③ Psperm integral Memb. protein (pH < 20/30)

Control of Epididymal Function

(1) Hormonal : ABP & SHBG can regulate the funct by conversion to DHT.

(2) Neural : Sympathetic Mediated (Sympathetic denervat → ↓ sperm Motility)

(3) Thermal: High temp. as in Varicocele / Cryptorchidism → Epid dysfunct.

What are the Epididymal Markers: (Not sure d.t variat in level even amongfertile Men)

- α-glucosidase
- L-carnitine
- Glycerol Phosphocholine.

Male Accessory Sex organs

- ~~Epididymis~~
- VD
- SV
- prostate
- Glands

((VD))

** . def:- 2 tubes or ducts Connecting the Rt & Lt epididymis to the ejac. ducts

** . Length : 35 cm

** . Start at : epididymal tail ⟶ enter the Inguinal Canal in the post. Part of Spermatic Cord ⟶ Int. ring ⟶ Pelvis < over the ureter / post. to " UB (med. to SV).

** . ends by enlargment & dilatation ⟶ Ampulla of Vas ⟶ join duct of SV ⟶ ejaculatory duct formation

** . Clinically it divided in to 5 parts:

1. Epididymal part : inside Tunica Vaginalis.
2. Scrotal " : outside Tunica (Site Vasectomy)
3. Inguinal
4. Pelvic part
5. Ampulla of Vas.

. Blood supply : Vasal a. (br. from Inf. vesical a. & br. from Int. iliac).

. Venous drainage: vasal veins.

Function: during ejaculation → (Reflex) Contraction of Smooth muscles → peristalsis → Expell of Spermatozoa to Urethra.

Clinical importance → Male Contraception

① Vasectomy (irreversible)

② Vaso occlusive Contraception (Reversible):

Vasal ← clips / plugs / valves → (Silicon plug block the vas)

③ Reversible inhibition of Sperm under Guidance: inject a gel material that Coat the Vas from inside → disturb the sperm during passage through the vas by unknown mech. ±

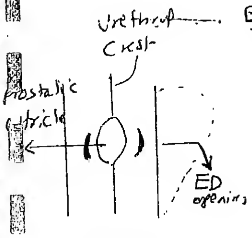
(RISUG) ← ③

1. Change of pH → Kill sperms
2. rupture of sperm memb. & disturbed sperm charge.
3. disturbe the -ve sperm discharge → prevent Zonal binding.

SV

Anatomy: 5-10 cm, 2 lobulated glands at post. lat aspect of UB (& lat. to Vas)

- Not felt by PR except if ⟨ inflammed or Obst.
- each gland has a duct & Join Ampulla of Vas $\xrightarrow{\text{NL}}$ ejaculatory duct (ED)



post. wall of Prostatic urethra

ED = 2 cm, open by slit-like opening on post wall of prostatic Urethra on the (Summit) of Urethral crest called (Verumontanum = Colliculus seminalis)

Funct^

(1) Semen Vol: its secret^ represent (60-80%) of Semen Vol; 2nd fract^ of split ejaculate.

(2) Alkalinity of Semen

(3) Coagulat^ & Viscosity of Semen
- d.t protein kinase = (Seminoglein I)
- d.t SV proteins (NL Semen is Viscous)

NB: Semen ejac. → liquid → coagul^ → liquefact^
- allow Contact of sperms c̄ ingredient of semen e.g ↑ motility, ↑chromatin stability & # Autoimmunity in ♀ tract.

So SV Hypofunct^ ⟨ ↓ Coagul^ : ↓ mot. ; ↑ Viscosity : ↓ mot & ↑chromatin stability

(4) Fructose Secretion:
- Androgen dependent
- ➜ Main Source of energy for sperms.
- NL level: 120 - 450 mg
- Abnlly ⟨ ↑↑ in: Azoo or oligo ; ↓↓ in: NL, PolyZoo, SV. obst or Absence, EDO

(5) Other Secretory (F)
(1) NO (↑ARt ↑ fructose) (2) production
- Vit C, Vit A, SOD
- (iii) Zinc (it ↓↓ of chromatin → allow decon... → NL fertili...)
- ↓ By ↑ Copper e.g Smoking.
- Dr. ...

Prostate

- **Anatomy**: Cone shaped pelvic structure that encircle the UB NECK & Urethra
- **Ass** → **Base**: at Bladder Neck
  Neck: at Superficial facia of urogenital diaphragm.

Functions

A. **Semen Vol.** represent 15-30% of Semin Vol, 1st Fract. of Split ejaculate

B. **Acidity** of Semen (PH 6.5) — Liquefaction < 30 min

C. **Secretory products**:

1. **Prostate Specific Proteins** = (Proteases = Fibrinolysins)
   - PSA (Seminin) — ↑ in Cancer prostate, PR, Prostatic
   - PAP (Prostate acid phosphatase)
   - PBP (Prostate binding Protein).

   Action → proteolysis (major function of prostate sec.) (Semen Liquefaction)

2. **Citric acid**
   - maintain osmotic equilib. of sperm
   - Motility
   - Ca ± Cit → prevent Calculi

3. **Spermin**: protein
   - Musk odour
   - Antibacteria

   Markers of:
   1. Epididymis
   2. SV
   3. prostate
   4. Testis

   Zinc, Fructose.

4. **Zinc**:
   - the main "Prostatic Antibact. factor" (PAF)
   - Sperm motility
   - Spermatogenesis
   - Chromatin Condensation
   - NL Test

NB
1. Conc. in Semen = 10 Conc. in Blood
2. Highest Conc. at sperm tail
3. Role in Chromatin decondensation

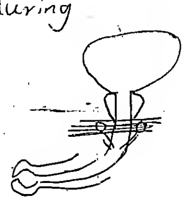
Glands.

- Cowpers (Bulbo urethral) glands
- Littre's (periurethral) "

def → one of Exocrine glands, present in the reproductive system of Human male (Homologous To ♀ Bartholin gland).

Site: Pair of pea-sized glands on (post. lat) aspects of membranous urethra [at base of penis] bet. 2 Layers of facia of urogenital diaphragm & (enclosed) by fibers of Ext. urethral Sphincter. Their ducts open into Bulbous Urethra.

- duct: 2.5 cm. excitation [illegible]
- Sec. → **Pre-ejaculate or pro semen** "during sexual Excitat^."
  "المذي"
  slightly
  - def: Clear, Colourless, viscous Fluid emits from urethra of males during sexual excitation.
  - Source: [mainly: Cowpers / ±: Littre's
  - Amount: variable (0-5 ml).
  - Composit^: ① Contain some chemicals of semen as acid phosphatase. → ++ Liquefact^
    ② Sperms → Controversy
    ↳ but ± Contain semen ē sperms from previous ejaculation that remains ... bulb.
    ④. Plasminogen activator → "Role in Coagulat^ (-- Coag)"



Function

1. Lubricate < urethra : for passage of sperms.
   penis : during sexual intercourse.
2. Neutrilize acidity of urine & Vagina.
3. Flush out any residual urine or FB
4. Pick up sperms remaining in urethra if bulb from the previous recent ejaculate. (Sa ± → Pregnancy).
5. Role in semen Coagulation. (Anti Coagulating effect of plasminogen Activator).

Medical problems related to it

1. may → pregnancy (Controversy).
2. Transmit HIV
3. overproduction may be a Complaint of many men, in Prostatorrhoea (O-D - sec. ass e defectat & urination & Not related To sexual Excitation as in Prosemen).

NL amount = 2.5 ml

4. Religious Attitudes: → Purification

Littres glands : present & open throughout penile urethra . secrete : Mucin

Physiology of sperm.

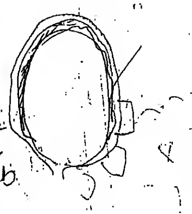
Structure → human sperm is ≃ 60 µm (30 µm) in Length & in contrast to other body cells it is flagellated & has (no) Cytoplasm

يتكون من ٣ اجزاء:

- Head (4.5 µm) [4.5] — Head [4.5, 3 µm]
- Neck (0.5 µm) (Connec [0.5] (40-70%)
- tail (55 µm) ← [55] µm

Head, oval in shape (4.5) µ in Length & 3 µm in diameter, Contains the nucleus that is surrounded anteriorly by the [acrosome] → ساترينه

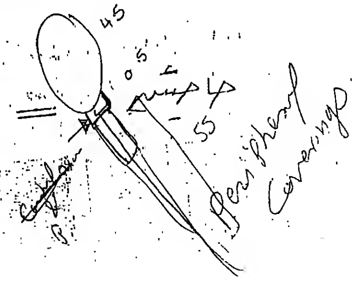
↓ modified bag or membrane bound organell that is formed by Golgi apparatus, Contains Lyzosomal enzs w necessary for ... it occupies the ant. (50-70%) of the sperm head infront of the nucleus.



Neck (Connecting piece) (Tail & Head) ... it is the post extension of sperm head that connects the head to the tail. (flagellum).

Tail formed of Central Core (called axonem) & peripheral coverings (w) are different from each piece of tail.

Axoneme (Central core)



Axoneme by F/M

2 Central Single Microtubules + 9 peripheral doublet Microtub.

↓ Surrounded by protein ring ω is attached to the peripheral Microtub. in a radial manner (radial spokes, links) like the wheel

↓ each of 2 Microt. of the 9 termed A & B

↓ each A Contains (2) arms (dynein arms) the outer one is large

the tail is divided In to 3 Pieces or areas.

Mid Piece → 5 um.
Main " → 45 um.
End " → 5 um.

a) The mid piece: its the area following the neck, the axoneme in this area is surr. by spirally arranged Mitoch. that act as energy source for the sperm. (5 um).

b) The Main principle piece: (45 - 50 um) the axoneme here is surr. by fibrous sheath & its devoid of Mitochond.

c) the End piece (5 um) this the distal end, the axoneme is not surrounded by either Mitochondria or fibrous sheath.

Mid Piece
Main
End
Central Core

① Mid piece → surr. by Mitoch. ✓
② Main → fibrous sheath ✓
③ End → no Mitoch. or fib sheath ✓